ELEMENTARY BACTERIOLOGY

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PREFACE TO THIRD EDITION

PROFOUND changes have been made for the third edition. Chapters on Yeasts, Molds, and Actinomyces; Bacterial Variation, Chemical Activities of Bacteria, and Influence of Chemicals on Bacteria have been added. The following chapters have been rewritten: Microorganisms Become the Allies of Man, The Phosphorus Cycle, Milk Products, Bacteria in Other Foods, and Bacteria as the Cause of Disease. Chapters dealing with the cycles of the elements have been condensed. Numerous changes, often with the addition of new material, have been made in the parts dealing with the founding of bacteriology, occurrence of bacteria, botulism, immunity, antitoxins and vaccines, infections common to man and the lower animals, and the filtrable viruses. The order of presentation of some of the material has been changed, in this way avoiding unnecessary duplication. Some of the old illustrations have been discarded and new ones added. Additions and changes have been great enough to necessitate again the resetting of the entire text. Yet, with all these changes, we have endeavored to maintain the original plan and size, which have received such a hearty welcome from so many teachers.

We are deeply grateful to our many friends and correspondents who have offered suggestive criticism for improving the volume. Especially are we appreciative of the helpful suggestions given by Dr. W. V. Halverson and Dr. K. R. Stevens. We hope this volume will continue to answer some of the questions raised by beginners and to inspire them with a desire for a more profound knowledge of the science which is lengthening man's life and making a better world in which to live.

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PREFACE

BACTERIA rule the world. Man is dependent on them from the day of his birth until the hour of his death. They are man's most useful servants and his most destructive master. It was they who first obtained a precarious foothold on the bleak primitive rock of early geological periods. Here they fed on the carbon dioxide of the air, the ammonia of the rain waters, and the traces of minerals which the water dissolved from the rocks. They acting in conjunction with physical and chemical agents eventually rendered the disintegrating rock a fit abode for higher plants. This paved the way for the higher animals and when they appeared bacteria settled on the skin and mucous membranes of their bodies. Most such bacteria perished, some became parasites while a few became progressive pathogens. These latter have become man's most destructive enemies and modern bacteriology deals to a considerable extent with their doings because there has been a never ending struggle between them and man to determine who shall possess the earth. They have defeated armies and destroyed cities. Nations which have been the glory of the world have gone down before them. They are no respectors of persons, nor are they governed by the edicts of rulers. However, at the command of modern science they are commencing to relinquish their hold, and some of earth's most fertile regions, where in the past the risk of death to the visitor was greater than a visit to the battle field, are being transformed into tropical health resorts. Still much remains to be done for the learning and the practicing of the laws of sanitation can prevent more suffering and save more lives than the abolition of war.

However, we should not lose sight of the fact that the great majority of all bacteria are innoxious and in many cases beneficial. Bacteria tear down the highly complex bodies of plants and animals from which the spark of life has departed, and salvage the parts so that they may be used again, thus restarting the elements on their wonderful constructive cycles. They help man in the

arts and industries, at first in a chance and haphazard way but as time went on man learned how to intelligently direct the action of the beneficial bacteria and also how to control the injurious microbes. This knowledge has resulted in a better world for man to live in, love in, rear his children in, and to die in at a riper age, because the knowledge gained is ever increasing man's expectancy of life.

In this book we hope is narrated in an elementary and interesting manner the doings of the beneficial and injurious microbes, and incidentally man's struggle to obtain mastery over his microscopic friends and foes. We have given briefly, but we hope not inadequately, information on classification and morphology requisite for an understanding of the important physiological actions of the interesting and important group of microörganisms known as bacteria. The meager consideration given to classification and morphology should not be taken to indicate that we depreciate their value but merely that we believe they should not be "ends but means" in an elementary course. Nor should the absence from this book of laboratory instructions be taken to indicate that laboratory work is not important. They are left for laboratory manuals.

The book has been written with the hope of furnishing a suitable text for courses in elementary bacteriology, yet it should prove of interest to nurses, home demonstrators, agricultural and home economic workers, and to that ever increasing multitude of readers who are turning from fiction to facts. For within the field of bacteriology one learns of curious, wonderful, marvelous and often beautiful specks of living protoplasm which have determined when, where, and how man may live. They have been more powerful than kings and potentates and have to a great extent moulded human history. In the subject are portrayed man's combat with invisible foes; combats which from the standpoint of heroism and determination rival military or even imaginary legends of antiquity, and as one reads one can remember that they are facts not fiction, and deal with the present not antiquity. It deals with a science in the making which today is calling for more Pasteurs, more Kochs, more Ehrlichs, more Lazears, and more Carrolls, who will conquer man's microscopic enemies and learn to guide his microscopic friends, for each bit of knowledge gained in this field carries man one step nearer the goal where he will be the master and not the slave of his environment.

To our many friends our hearty thanks are offered for the valuable encouragement and assistance given in the preparation of this book. We are especially happy to acknowledge the many valuable suggestions we have received from Dr. John A. Widtsoe, Dr. T. L. Martin, Dr. H. J. Frederick, Professor C. T. Hirst, and Fred Hammerly who have read parts or all of the manuscript. Nor do we forget the assistance rendered by J. Dudley Greaves in preparing many of the illustrations and to Claude Zobell, J. Dudley Greaves, and Florence D. Greaves for help in much of the routine work. We are also deeply grateful to Mrs. Blanche C. Pittman and Miss Maida Muir for their painstaking care in the preparation of the manuscript for the press.

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CHAPTER I

INTRODUCTION

One autumn day I was seated in a cafeteria when a party of six entered. They served themselves and proceeded to a table nearby just vacated by another party. One of the members took from his pocket a handkerchief and brushed the crumbs from the table. Soon the party was enjoying their midday meal. I had known at college the young gentleman who had so nicely dusted off the table. He was a brilliant man, well trained, and had meritoriously gained his Ph. D. He had incidentally come into possession of T. B.

Some time later I stopped at a hotel with two other college men, both of whom had received their advanced degrees from leading institutions of the country. I noticed that each carried his tooth brush and conscientiously used it each night and morning. One of them placed his moistened brush on the edge of the washbasin while he searched for his tooth paste which when found was used with his polluted brush.

These two incidents, together with many others of similar nature, have often caused me to ask myself the question: In this day when health work has shifted from the environment to the individual and when each individual can do so much to prevent infection, are individuals prepared for life in our modern, intimate, and complex society without learning the a b c's of sanitation? The few fundamentals that the physician uses when visiting cases of communicable diseases and the same principles that have made it possible for the trained attendant to nurse infectious diseases without contracting them herself or conveying them to others can be learned and used by all.

Fundamentals.—Today we smile as we read van Helmont's formula for producing mice: "Place some dirty rags together with a few grains of wheat, or a piece of cheese, in a dark place

and in a few days they will be transformed into mice." The same philosopher's method of engendering scorpions is amusing to us: "Scoop out a hole in a brick, put into it some sweet basil. Lay a second brick upon the first so that the hole will be imperfectly covered. Expose the two bricks to the sun, and at the end of a few days the smell of the sweet basil, acting as a ferment, will change the herb into a real scorpion."

How many are there today who believe that disease germs breed in stagnant water, deserted lots, decaying vegetables, and other bad surroundings? What percentage of humanity today knows that disease-producing micro-organisms are always the descendants of other similar disease-producing micro-organisms, and if these are kept away from the body of man that there never can develop within him an infectious disease be his physical condition ever so bad? These micro-organisms always breed true, and it is just as possible to grow a crop of diphtheria germs, typhoid germs, or tuberculosis germs as it is to raise a crop of potatoes; and the bacteriologist would be just as surprised to reap a crop of diphtheria where he planted typhoid as the farmer would be to harvest potatoes where he had planted corn.

Special plants require special soil and definite conditions of temperature, moisture, and aeration for their growth. The medium in which the disease-producing organisms grow is with few exceptions limited to man and the lower animals; hence, when seeking these specific types of organisms for laboratory study, we turn to man and the things which he has handled.

The evidence is complete that the causative agents of tuberculosis, pneumonia, influenza, cerebrospinal meningitis, typhus fever, smallpox, whooping cough, gonorrhea, syphilis, malaria, yellow fever, and sleeping sickness multiply only in the body of animals. It is also an established fact that the number reaching the body of one animal is no larger than the number that leave the body of another animal.

Diphtheria, which for so long has been considered a filth disease, that is, the germs were supposed to have a habitat outside of the body in various forms of dirt, is now known to be a purely communicable disease. The organism is more resistant than are some other disease producers, but there is as yet no evidence to show that it is propagated outside the body except occasionally in milk.

Whereas it is well known that water occasionally gives rise to

typhoid fever, it is also well established that typhoid bacteria rapidly disappear from water and probably never live in water longer than fifteen or twenty days. There is no reason for believing that the germ ever multiplies in water. Hence, water requires a constant source of new infection from the body of a human individual to be at all dangerous, for it is evident that while the typhoid bacillus may live for some time in the soil there is no evidence that it can multiply in soil. This organism, then, along with many others, has its origin only in man. Therefore man is man's greatest enemy so far as the production of disease is concerned.

From time immemorial vapors and emanations, gaseous or otherwise, have been considered to be frequent causes of disease. But with the growth of the subject of bacteriology it was found that bacteria were the real cause of disease. Since then, even among many educated persons, the favorite explanation of the transmission of disease has been that diseases are conveyed in the air. Experience has taught that even smallpox or measles can be housed in the same hospital or even in the same wards without danger provided the necessary precautions are taken to prevent the carrying of infection from one individual to another by the attendants.

This gives rise to the question: How are the various disease-producing bacteria spread?

Some of them use the common housefly as their airship, others the flea, and still others chance a ride with the bedbug. At other times they find their way into food, or into clothing and are transported from place to place in this manner. Some are picked up with the particles of dust, but to the disease-producing organisms this method of travel is highly fatal, for when struck by the direct rays of the sun they are soon killed. Many could not make a long journey on dust particles even in the dark for the bodies of the organisms are about 90 per cent water, and if not clinging to some moist substance they soon perish.

Numerous disease-producing bacteria are found in the mouth of apparently healthy individuals, and it is a matter of little difficulty for these organisms to journey from the lips of one individual to the lips of another. The ordinary drinking cup is only one of the hundred and one things which come in contact with the mouths of many individuals every day, and serve as the means of transportation.

Then again, "our fingers are ten swabs which are continually

picking up all sorts of dirt from door knobs, stair rails, car straps, counters, chairs, books, money, water closets, our own shoes and rubbers, and other things, the list of which can be extended almost indefinitely. Visible dirt gathers quickly. With the visible dirt is also gathered all too frequently some of the secretions from another person. Often these secretions contain the germs of tuberculosis, diphtheria, meningitis, or scarlet fever. Watch again how often the fingers go to the lips. Unfortunately these finger swabs, unlike the cotton swab of the physician, plant their germs not in the test tube but on human mucous membrane."

Hence, while the three R's may be fast slipping from the first place in our educational curriculum, the three F's—fingers, flies, and food—reign supreme in sanitation; and the individual who has these facts well grounded has a powerful weapon with which to protect himself and others.

We would laugh to scorn the individual who today would advocate keeping in the prime of condition, so that he might resist the bullets of the enemy. Yet, how many believe that general good health protects against infection? We need only use our eyes to become aware of the fact that the physically fit and robust fall prey to typhoid fever, smallpox, influenza, and most other infectious diseases as well as the weaklings.

One should keep in the best of physical condition, for it is not until then that life is really made worth living; but everyone must learn that disease-producing organisms must be kept away from his body if he wishes to avoid infection. This implies a knowledge of where these micro-organisms grow, how they are carried from their place of growth, and how they enter the body. In some cases where it is impossible to keep them out of our bodies the situation becomes grave, unless we have within us the specific antidote that science has discovered against them. This is especially true in respect to smallpox, typhoid, diphtheria, tetanus, and a number of other diseases. Too often do we find even educated individuals opposing the use of these antidotes because of the danger they believe to be associated with their use when as a matter of fact the individual who is vaccinated, given an antitoxin or a toxin-antitoxin is not endangering his life or limb as much as the person who takes a motor ride.

Facts in Disease Prevention.—We have certain fundamentals in the prevention of disease that should be the common knowledge of every boy, or girl, man or woman.

1. Micro-organisms are the cause of the communicable diseases and these are the descendants of other similar micro-organisms.

2. The overwhelming majority of all micro-organisms which cause disease in man multiply only in the body of man or the lower animals.

3. Diseases are transmitted through direct contact or through the intervention of insects. In other words, the principal carriers of disease are fingers, flies, and food.

4. A high state of bodily health should be the aim of all, but this alone will not confer immunity to the communicable diseases. Such immunity may often be conferred by the vaccines, antitoxins, and toxins-antitoxins.

Moreover, it should be understood that there is an ethical side to the subject, for whereas the United States constitution guarantees to each of us life, liberty, and the pursuit of happiness it does not give us the right to infect another carelessly. All individuals are willing to wage a relentless war on flies, mosquitoes, and the lower animals, so that these will not carry disease to man, yet they are not willing to remain from the theater when suffering with a cold, to refrain, when feeling indisposed, from handling the food or milk to be used by others, to see that the milk they are selling to their neighbor, or feeding to their own children is free from disease germs.

The relentless war that has been waged against lower animals which convey disease to man is causing cholera, plague, and yellow fever to disappear from the face of the earth, but no such success has attended the directly communicable diseases as some of these are on the increase. Yet our knowledge at the present time is sufficient to stamp out certain diseases that are on the increase if each would but learn that he is his brother's keeper.

What a glorious attainment it would be if everyone could have firmly rooted in his nature this slogan: I am building for myself and future generations; hence, the structure which I rear must be built on a firm foundation. For the foundation I shall use *Character*, for the building stones *Health*, and for the cement *Education*. The building stones I wish free from blemishes and defects, and in order to keep them so I shall learn and live the laws of sanitation.

Can Be Learned by Young.—That these principles can be learned by the very young is shown by the following: A five-

year-old boy, who had watched the growth of disease-producing bacteria in media and had seen the methods of destroying them, found a bell which he wished to present to his baby sister. His mother's attention was first directed to it when the boy attempted to get the bell from the oven where it had baked for several hours so it would be a fit plaything for sister. When the baby sister was presented with the bell it had been sterilized as effectively as could have been done by the best physician.

When the baby sister became old enough to enter school a teacher stopped her father on the street one day and said: "We had a very amusing experience in school today. When the doctor was examining your little girl he asked her to open her mouth, but as his hand moved toward her mouth she closed it, and opened it only when commanded to do so, closing it again every time the hand of the examiner moved toward it. This was repeated a number of times until between sobs she looked at the doctor and said, 'You can't put your finger in my mouth; it's got germs on it and they will make me sick.'" Not only can these principles be learned by the young, but they should be made an intimate part of their education. To be sure they are only children, but the children of today will be the future fathers and mothers and they in turn will nurse any communicable diseases that may be present within their families, and pass them on to others and to their children's children.

Deals with the Interesting.—There is no subject which has a wider appeal than bacteriology. If one is of a mathematical turn of mind he can apply his knowledge of geometrical progression to the problem of the multiplication of bacteria. If bacterial multiplication went on unchecked the descendants of one cell would in two days number 281,000,000,000. What would it be at the end of a month? What would be the weight in tons of the resulting mass when one cell weighed one-hundred millionth of a milligram? These are some of the problems that one might wish to solve.

If one loves the beautiful and the curious, nothing can be found that is more fascinating than the study of pigmented bacteria whose bodies contain the most remarkable varieties of colors. In this connection an explanation for many of the weird stories of ghosts and goblins can be found, particularly in the study of the light-producing fluorescent bacteria which often cause meat or decaying stumps to shine at night with an uncanny light.

If one is interested in stories of heroism, they are to be found in the history of bacteriology as in no other place in the annals of history. Should anyone doubt this let him read the life of Pasteur during the time he was studying rabies, the history of the conquest of the plague, the story of the work of the Yellow Fever Commission which made possible the digging of the Panama Canal. Here one can follow men in their valiant struggles against an invisible foe. One can follow the dark and anxious hours of sorrow and suffering before the causes of the various diseases were found. Then one may read the inscriptions on the tablets such as the one erected to the memory of Dr. Lazear who gave his life in the fight against Yellow Fever, and which reads: "With more than the courage and devotion of the soldier, he risked and lost his life to show how a fearful pestilence is communicated and how its ravages may be prevented."

If one wishes to see examples of great determination these can be found in the lives of men like Ehrlich, who undertook to find a curative agent for one of man's most horrible diseases, syphilis. The first compound prepared and tested was labelled No. 1. It was found to have no value. The second, No. 2, after having been carefully prepared and tried out was no better. This process was repeated six hundred and five times, each being a failure. The compounds often required weeks of careful, patient work to prepare, but the determination of this man was not to be hampered, and with the six hundred and sixth trial Ehrlich was able to give mankind the cure for the third great plague.

That man has become a master at killing his fellowmen was shown by the late war, but his works of destruction seem like child's play when compared with the destruction of bacteria. If one doubts it let him read the history of influenza, and he will find that it alone killed more human beings in two short years than man in four. Disease claimed four times as many lives during the Spanish-American War as did the enemies' bullets. During the nineteenth century man slew on the battlefield 19,000,000; yet, the little tuberculosis organism during the same time slew 34,000,000. Napoleon slew his tens of thousands, but the one discovery of Lister has saved more than this from the ravages of bacteria.

The individual interested in engineering achievements may have admired men's ingenuity in the fixing of atmospheric nitrogen by means of a gigantic arclight in a chimney through which is blown a current of hot air. The flame of this arclight has

a diameter of 7 feet and reaches a temperature of approximately 3500° C. The resulting product created by the flame when dissolved in water gives us nitric acid. In still another method, air is cooled to —194° C., the nitrogen boiled off, mixed with hydrogen in the proportion of 1 to 3, heated to a temperature of 550° C., and this when passed under an extremely high pressure through a catalyzer electrically heated to about 550° C. gives as a result ammonia. And yet, how crude and clumsy are these methods when compared with the engineering feats of the bacteria, in their fixation of atmospheric nitrogen.

It is thus evident that bacteriology is a subject which is of immediate practical value to everyone and not merely a preparation for something that may be undertaken in the distant future. It is a subject that teems with interest and touches alike the everyday life of the one who returns to the shop, the office, the farm, or the home.

CHAPTER II

FOUNDING OF BACTERIOLOGY

AEONS ago bacteria gained a precarious foothold on bleak, primitive rock and manufactured acids which in time produced an abode fit for the growth of higher plants. Walcott claims to have found fossil bacteria in geological formations, the age of which is estimated to be 33,000,000 years. Even the sluggish, heavily armored dinosaurs were tormented and often fell prey to bacterial infections, as is shown by the fossils recovered from Permian deposits. From these fossils it appears that the same diseases tormented the early geological animals as attack the animals of today. However, we must remember that it is the diseases of the skeleton and not the vital organs which have left their records in the rocks. Be this as it may, we are certain that bacteria often ended the life of the ancient as well as the modern animal; and it was they who quickly removed in most cases the bodies of the dead plants and animals. Although bacteria were not known to man until the perfecting of the compound microscope in the last quarter of the seventeenth century, yet they manifested themselves centuries before. As early as the dawn of written history the putrefaction of meat, the fermentation of fruit juices, the decay of wood, and the sickening of animals were recorded, and numerous and interesting are the theories that were evolved to account for the observed facts.

Theories of Disease.—The ancient pagans accounted for disease in their theory of creation. Prometheus, to whom was entrusted the creation of man and the task of providing him and other animals with facilities necessary for their preservation, was, as the legend runs, so liberal in providing for the lower animals that when man was reached nothing remained to bestow upon him. Man, naked and empty-handed, was left unable to compete with the lower animals; hence, man was given fire stolen from Jupiter with which to shape weapons. For this theft Pandora was sent as a punishment to Prometheus and his brother. In the house of these brothers was a jar in which

were kept all noxious articles that had been left over after the fitting of man for his new abode. Pandora in eager curiosity slipped off the cover, and out rushed the various plagues which afflict mankind.

The active imagination which caused the children of the race to fill the woods with fauns and satyrs, and the streams with nymphs and naiads, naturally caused them to see in the sick the actions of demons. The Babylonians regarded disease as "the work of demons which swarmed in the earth, air, and water." These were supposed to enter the body of their victim and rule in a merciless fashion. "The possessed man tossed and shaken in fever, pained and wrenched as though some living creature were tearing or twisting him within, rationally finds a personal spiritual cause for his sufferings. In hideous dreams he may even see the very ghost or nightmare fiend that plagues him. This is the savage theory of demonic possession, which has been for ages, and still remains, the dominant theory of disease and inspiration among the lower races. It is obviously based upon animistic interpretation, most genuine and rational in its proper place in man's intellectual history, of the actual symptoms of the case."

Such was the belief concerning disease among the early ancestors of modern civilized man, and such is the belief of primitive man even today. Logically, such a disease could be cured only by dislodging the invading demon. And what was more natural than to conclude that this could be accomplished either by force or by flattery? The medicine man attempted to force out the invader by making the habitation unbearable. The victims were whipped, loaded with chains, given ill-smelling and bad-tasting drugs, all in the hope that the demon would seek more congenial quarters. Often attempts were made to entice him out. Here charms, incantations, and sacrifices played a prominent part. For centuries roots and herbs were prescribed because of their symbolic form. They used curious things such as the moss scraped from the head of a culprit hanged in chains, or from the Egyptian mummies, or the roots of plants gathered in a graveyard during the dark of the moon. It was not until man discarded the belief that insanity is a manifestation of demonic possession that the last vestige of the demonic theory disappeared from the medical idea of civilized man.

A doctrine quite as old as the demonic theory is that all the joys and ills of man are determined by the position of the heav-

enly bodies, and even in modern times no less an authority than Noah Webster wrote a book in which he attempted to prove that epidemics are due to earthquakes and other terrestial disturbances. Had he written after the great San Francisco earthquake he could have used this as an example, for plague occurred after the earthquake. But what was the cause? In all probability the plague had existed in a sporadic form in San Francisco's "Chinatown" and other places on the Pacific Coast for many years. "Chinatown" was more or less isolated, but

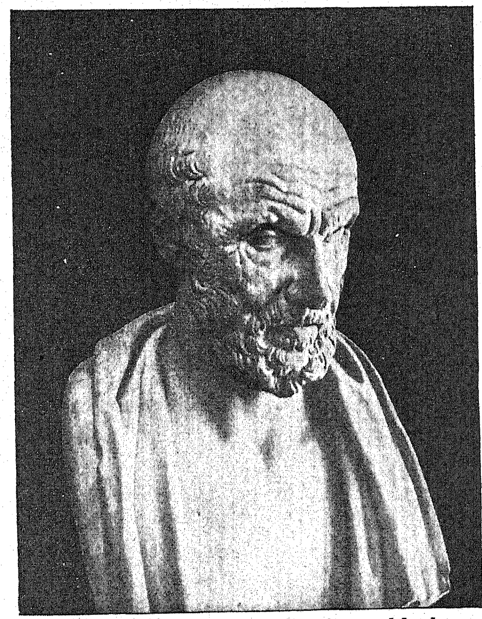


Fig. 1.—Hippocrates (460–370 B. C.). (*Greek marble bust in the British Museum.*)

due to the earthquake the inhabitants of the locality were scattered through the city and with them also the plague-infected rats and fleas. Hence, there was a flare-up of the plague, which, had it occurred during the Dark Ages, would have spread like wildfire.

Hippocrates, the father of medicine, taught in the fifth century B. C. that disease is due to a pestilential condition of the air. Gaseous substances were supposed to emanate from the soil, stagnant pools, marshes, decaying and putrescent substances,

and crowded habitations. He vaguely realized that there was a relationship between water and disease for he advocated the boiling of drinking water.

As a result of such teachings, aqueducts and sewerage systems were constructed by ancient Rome. The famous Cloaca Maxima, constructed in the sixth century B. C., ran from the Forum to the Tiber and drained the public latrines and those of private homes adjoining it. Some of the great aqueducts with their accompanying filters and settling tanks built at this early period are still to be found at the present time. That built by Quintus Marcius was 56 miles in length. However, the people of later periods did not accept these theories of disease, and during the Dark Ages they were replaced by those of mysticism and the officials boiled witches instead of water to prevent disease. The result, obviously enough, was that sanitation gave way to filth and pestilence.

Mysticism also entered into the teachings of Hippocrates, for he taught that the body contains four humors: Blood, phlegm, yellow bile, and black bile. When these were present in the right proportion, one enjoyed good health. Small variations accounted for differences in temperament; a sanguine temperament was due to a preponderance of blood, a phlegmatic temperament to an excess of phlegm, and a bilious or melancholic temperament to a surplus of bile. Greater variations resulted in illness; hence, it was the object of the medical profession to keep the proper balance of the humors and in this way maintain health.

These latter theories of Hippocrates were not questioned until the seventh century, when a flood of new theories burst forth. Some of these were even more complex and mystical than the ones they were intended to supplant. Disease was regarded as "an intestinal movement of particles," "a struggle between nature and disease-producing matter," "a lack of tone or an insufficient amount of tone." There was the dynamic organic system of Stahl, who believed that the soul was the supreme principle of disease. There was the mechanico-dynamic system of Hoffmann, which stated that life expressed itself in motion, and that all manifestations in the body are controlled by nervous spirits. The school of Montpelier taught that various organs possess individual life. Mesmer claimed that magnetic fluids flow from the hand, and the Bremontau system asserted that all that was necessary for a cure was to determine the grade of a disease in

accordance with the strength or weakness of the active irritation and to adjust the right proportion of strengthening or weakening medicine to the case.

The discovery of bacteria and the proof that they are the cause of the communicable diseases were slowly and surely to replace this confusion of mysticism and superstition by a rational and effective method of preventing and curing disease.

Discovery of Bacteria.—The germ theory of disease was probably evolved in the mind of an occasional genius many centuries ago, but it remained a theory until a comparatively recent date. It is possible that the Roman Catholic monk, Kircher, saw bac-

Fig. 2.—Athanasius Kircher (1602–1680).

Fig. 3.—Antonj van Leeuwenhoek (1632–1723).

teria in 1671. He examined water, decaying matter, blood, and pus. In these he found what he called "invisible worms." He even speculated concerning the relationship between these "invisible worms" and disease. Some bodies described were undoubtedly chains of blood corpuscles and the description of others is so inexact that it cannot definitely be stated that he saw bacteria.

Be this as it may, we know that bacteria were seen and accurately described soon after in 1683 by a Dutch linen draper, Antonj van Leeuwenhoek who is often called the father of bacteriology. During the course of his life he contributed to the

British Royal Society approximately 200 papers dealing with various scientific topics. He also manufactured scores of microscopes, many of which were superior to any that had been made previously. With these he examined various things—raindrops, saliva, and putrefying substances. He found in every case living, moving animalcules which prior to his time had been unrecognized. We can imagine his joy and surprise from his statement: "I saw with wonder that my material contained many tiny animals which moved about in a most amusing fashion; the largest of these, *A* (Fig. 4), showed the liveliest and most active

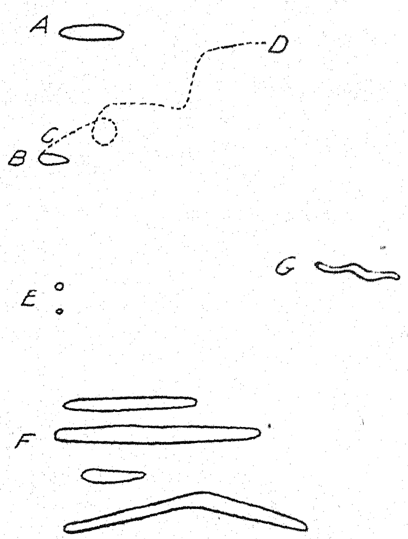


Fig. 4.—Drawings of bacteria. The dotted line *C-D* indicates movement of the bacteria. (*After Leeuwenhoek.*)

motion, moving through the water or saliva as a fish of prey darts through the sea; they were found everywhere, although not in large numbers. A second kind was similar to that marked *B* (Fig. 4) which sometimes spun around in a circle like a top. These were present in larger numbers and sometimes described a path that is shown in *C* to *D* (Fig. 4). A third kind could not be distinguished so clearly; now they appeared oblong, now quite round. They were so very small that they did not seem larger than the bodies marked *E*, and besides they moved so rapidly that they were continually running into one another.

They looked like a swarm of gnats or flies dancing about together. I had the impression that I was looking at several thousand in a given part of water or saliva mixed with a particle from the teeth no larger than a grain of sand, even when only one part of the material was added to nine parts of water or saliva. Further, the greater part of the material consisted of an extraordinary number of rods, of widely different length but of the same diameter; some were curved, some straight as shown in *F*; they lay irregularly and were interlaced. Since I have previously seen animalcules of this same kind in water, I endeavored to observe whether there was life in them, but in none did I see the smallest movement that might be taken as a sign of life." Some of them he considered traveled with the speed of lightning and even "tore through each other."

This patient worker, supplied with a microscope of his own make, gave a fairly accurate description of these minute forms of life. However, it did not awaken the world to even a faint realization of the marvelous invisible forms of life which were present in everything either as a blessing or as a curse. It did, however, revive a discussion which waxed long and furious as to whether life can spring spontaneously from dead matter or is always the offspring of preexisting parents.

Spontaneous Generation.—Theories as to the origin of life are as old as the human race. The doctrine of spontaneous generation is one of them. The race is like a child in that during the early stages of development the imagination is the predominating instinct. We thus find the first descriptions of the origin of life highly imaginative. The Greeks looked on the Goddess Gea as the mother of mankind. In their glorious mythology they pictured men and women as springing into life from the stones cast on Gea. The Celts pictured the soil as peopled with gnomes and pixies, friends or enemies of mankind. Many ancient writers fancifully portrayed the transforming of dead into living matter. The Greek philosophers taught it. Aristotle wrote in 384 B. C.: "Animals sometimes arise in soil, in plants, or in other animals."

Three centuries later Ovid, in his dissertation on the pythagorean philosophy, defends the doctrine of spontaneous generation, while Vergil (70–19 B. C.) in the Georgics, gives directions for the artificial production of bees.

Paracelsus (1493–1541), the Swiss medical philosopher who greatly confused fact and fancy, gives instruction for the making

of homunculi. Certain substances are to be placed in a bottle; the bottle well stoppered and buried in a dung heap. Every day certain incantations must be pronounced over the bottle. In time, so Paracelsus declares, a small living human being (homunculus) will appear in the bottle. However, he naïvely admits that he never succeeded in keeping the homunculus alive after taking it from the bottle. Kircher even went a step further and described and pictured certain animals which he claimed were spontaneously produced before his eyes through the influence of water on fragments of plants. Cardan (1501–1576) thought that water gave rise to fish and animals and that it was the cause of fermentation.

Fig. 5.—Paracelsus (1493–1541).

An Italian, Bononani, tells of a wonderful transformation which he claims to have witnessed. Rotten timber which he rescued from the sea produced worms; these gave rise to butterflies; and strangest of all, the butterflies became birds and flew away. Gradually these grotesque fanciful opinions concerning spontaneous generation were abandoned, and it was believed that only the lower plants and animals, seaweeds, algae, lichens, lice, mites, and maggots, could develop spontaneously. Even today we can find fairly intelligent individuals who believe that mites and lice can develop without parents and that the hair from the tail or mane of a horse will change

into a worm or snake if placed in water and exposed to light and warmth.

Everyone took it as a self-evident fact that maggots originated spontaneously from decomposing meat or cheese until an Italian poet and physician, Redi (1626–1697), took the simple precaution of screening the mouths of large jars containing meat so that flies could not enter. The flies attracted by the odor deposited their eggs on the gauze, and it was from these eggs that the so-called "worms" arose.

By the middle of the sixteenth century the theory of spontaneous generation of mice, scorpions, and maggots had been proved untenable. But the problem was far from being settled

Fig. 6.—Francesco Redi (1626–1697).

as regarded other forms of life, for when Leeuwenhoek with the aid of his lenses discovered the various living and moving animalcules in raindrops, saliva, and many putrefying substances, it was at once believed that the riddle of life had finally been solved. Now anyone provided with the microscope, could easily demonstrate for himself the spontaneous generation of microscopical eels in vinegar, or produce myriads of different and interesting living creatures in simple infusions of hay or other organic material.

Origin of Bacteria.—Following the discovery of bacteria, theories became rife as to their origin. Two champions of the theory of spontaneous generation who now took up the con-

troversy were Needham and Buffon. Needham, a Catholic priest, evolved the theory that a force called "productive" or "vegetative" existed which was responsible for the formation of organized beings; while the great naturalist, Buffon, elaborated the theory that there were certain unchangeable parts common to all living creatures. After death, according to his conception, these ultimate constituents were set free and became very active, until with one another and still other particles they gave rise to swarms of microscopical creatures.

Needham experimented somewhat and in 1745 took decaying organic matter and enclosed it in a vessel. This he placed upon hot ashes to destroy any existing animalcules. On examining the contents of the flasks he found micro-organisms which had not been there in the beginning. Later in 1769 Spallanzani repeated the same work, for he felt that Needham had not exercised sufficient care and that the organisms entered from the outside. Spallanzani boiled the material for one hour and kept it in hermetically sealed flasks. He wrote: "I used hermetically sealed vessels. I kept them for one hour in boiling water, and after opening and examining their contents, after a reasonable interval I found not the slightest trace of animalcules, though I had examined the infusion from nineteen different vessels."

But the believers in the theory of spontaneous generation were not convinced. They claimed that boiling altered the character of the infusion in such a manner that it was unable to produce life. Voltaire, with his characteristic satire, took up the fight at this point and ridiculed the operations of the English clergy "who had engendered the eels in the gravy of boiled mutton," and he very wittily remarked: "It is strange that men should deny a creator and yet attribute to themselves the power of creating eels." But this was a controversy to be settled not by ridicule but by experimental evidence. Spallanzani answered his opponents by cracking one of the flasks so that air could enter. Decay soon set in. This, however, was not sufficient to overthrow the popular belief, for the claim was made that in the sealing of the flasks air was excluded, and air was essential to the generation of these forms of life. This objection was taken up by a number of very ingenious investigators. Schulze, in 1836, passed air through strong acids and then into boiled infusions and failed to find any living organisms in the infusion, whereas Schwann passed the air through highly heated tubes with the same results. These methods were criticized by

their opponents who claimed that the chemical alteration of the air subjected to such drastic treatment had been responsible for the absence of bacteria in the infusion. The work of Schroeder and Dusch (1853) was more convincing, for they found that it was sufficient to simply stopper the bottles with cotton plugs; the air passed in, but the micro-organisms were held back by the cotton and the contents of the flasks kept free from contamination.

Even this was not sufficient to overthrow the belief of spontaneous generation, for as late as 1859 Pauchet revived it in a book in which he heaped experiment upon experiment and argument upon argument spiced with logic and sarcasm in favor of spontaneous generation. In this he was opposed by Pasteur who collected the floating particles of air surrounding his laboratory in the Rue d'Ulm and subjected them to microscopical examination. Pasteur sowed these particles in sterilized infusion and obtained abundant crops of microscopical organisms. He repeated and confirmed the experiments of Schwann which had been contested by Pauchet. He showed that the cause which communicated life to the infusions was not uniformly diffused, that in the workshops and crowded streets of Paris living organisms were numerous, whereas on the tops of high mountains and glaciers the air was usually free from life. He showed that beef tea sterilized in flasks with the neck bent like that of a swan did not spoil even though exposed to the atmosphere. As late as 1922 there was exhibited in the United States one of these flasks of beef tea which it was claimed Pasteur had prepared over fifty years before; it was still clear and free from life.

Nearly all Paris was at his famous lecture at the Sorbonne April 7, 1864, in which he portrayed vividly and with a touch of scorn for his adversaries his conclusions concerning the origin of bacteria. He said: "There is no condition known today in which you can affirm that microscopic beings come into the world without germs, without parents like themselves. They who allege it have been the sport of illusions, of ill-made experiments, vitiated by errors which they have not been able to perceive, and have not known how to avoid." In a passage of singular beauty he described himself watching and imploring his flasks to give him a sign of life, but they would not—"for I have kept from them, and am still keeping from them, that one thing which is above the power of man to make; I

have kept from them the germs which float in the air, I have kept from them life."

In England the controversy was waged between Bastian and Tyndall. The latter proved that in an atmosphere devoid of dust, as on the tops of mountains and in some ingeniously constructed boxes used by him, perishable substances such as beef tea, if sterile when placed in such an atmosphere will keep for an indefinite period. However, every now and then he found that the contents of a flask would spoil, even after it had been carefully stoppered and boiled. This remained a stumbling block in the way of those who maintained that life sprang only from life until the year 1865 when Pasteur demonstrated that many bacteria may pass into a resting stage in which they can withstand conditions that quickly kill them when in the vegetative form. Eleven years later Cohn of Breslau investigated very carefully organisms in this resting or spore stage, and today we know forms of micro-organisms that are able to withstand boiling water for sixteen hours without being killed, and others even resistant enough to endure for many hours a 10 per cent solution of carbolic acid.

Thus was established the principle that life springs only from life, from the viewpoint of the welfare of the human race, the most momentous discovery ever made by man, for on it are reared those three sciences which have done so much to prevent, alleviate, and cure human ills: Bacteriology, pathology, and surgery.

Early Classifications of Bacteria.—Having discovered new objects, it is human nature to ask: From whence did they come? What are they? How shall we classify them? We have seen that it required two hundred years to answer the first question and during all this time and even at the present time there are efforts being made to answer the others. Leeuwenhoek believed that the microscopical forms of life which he had discovered were animals, and inasmuch as they grew in various infusions, he called them "infusion-animalcules." Although he gave no specific names and his description is often faulty due to his crude microscope, it has been stated that he distinguished twenty-seven kinds or "species" of his infusoria.

Linnaeus (1707–1778), the great Swedish botanist, in his classification of plants, with what appears to be a little outburst of temper, assigns these minute organisms to a division of his great class "vermes" and gave to them the name of

"chaos." O. F. Müller (1730–1784), the Danish naturalist, was the first to attempt to bring order out of this "chaos." He figured and named about a hundred more organisms than had been described by Leeuwenhoek. He used the linnaean binomial nomenclature. The genus Vibrio comprised that strange mixture of species which we today know as bacteria.

Fifty years after the appearance of Müller's work, Ehrenberg published a magnificent volume on the "infusion-animalcules." In this he had 1500 exquisite drawings of various organisms. This really marks the first serious attempt at classification, and in it appeared numerous names which have been retained even to the present time. Here for the first time appeared the name bacterium, and from it has originated the name bacteriology, the name of the science dealing with these microscopical forms of plant life.

There appeared almost simultaneously the work of Schroeder and Cohn. The work of the former dealt with the pigment-producing bacteria and their growth on potatoes, thus giving to the subject a new cultural medium. Cohn divided the microscopical organisms into two classes: The one comprising those multiplying by budding and the second those multiplying by fission. To the organisms belonging to the second class he gave the name bacteria and placed them in the plant kingdom. He further classified bacteria into four tribes, and there appear in this classification generic names which are used even today in much the same manner as used by Cohn.

Since the time of Cohn many changes have been made in the classification. New ones have been proposed, many with meritorious parts but all with defects; and today, even though scientists have come nearer to the coveted goal, no classification has yet been devised that has proved satisfactory enough to be universally accepted.

Development of Methods.—The advancements in bacteriology have been intimately associated with the development of the microscope. The writings of Pliny, Plutarch, and Seneca make reference to simple magnifiers which probably were used in making the fine engravings found on ancient gems. During the early years of the fourteenth century lenses were used by ingenious Italians to improve failing sight, but the discovery of the principle underlying the compound microscope was made by Johann Janssen and his son, Zacharias, during the latter part of the sixteenth century. But the manufacture of the first compound

microscope is usually attributed to Robert Hooke in 1665. This microscope was crude and did not give a clear image; hence, Leeuwenhoek used simple lenses which he himself ground into proper shape and curvature and mounted between two perforated pieces of silver. Greater magnification, however, was desirable, and various efforts were made to improve these crude instruments. It was due primarily to the efforts of Joseph Jackson Lister that the compound microscope became a prac-

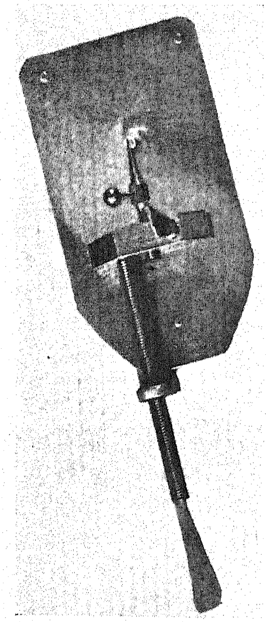


Fig. 7.—Van Leeuwenhoek's microscope. (*After Gustav Fassin, Scientific Monthly.*)

tical working instrument. He, with the aid of the celebrated optician Tulley, devised means for the combination of lenses of crown glass with others of flint glass so that the refractive errors of one were corrected by the other. With such an instrument it was possible to get a highly magnified image free from distortion and fringes of color that had heretofore been such a drawback in the use of the compound microscope. The perfection of the modern microscope was reached only during the

latter part of the nineteenth century. The more important features of which are: (1) The achromatic lens system; (2) water and oil immersion lenses; (3) the apochromatic objectives; (4) the compensation eyepiece; (5) substage illumination and the Abbé condenser; (6) the control of focus with micromillimeter screws; and the mechanical stage. The microscope of today gives a magnification up to 2500 to 3000 times with clear definition. It has become a vital factor in the advancement of bacteriology.

We have found the two great landmarks in the history of bacteriology to be the discovery of bacteria by Leeuwenhoek

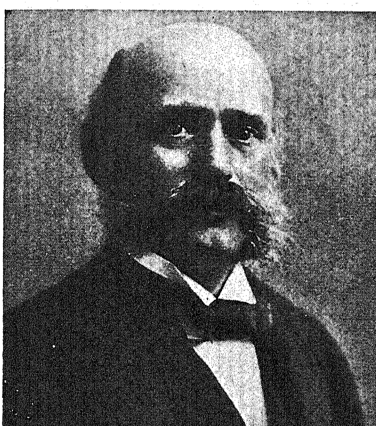


Fig. 8.—Carl Weigert (1845–1904).

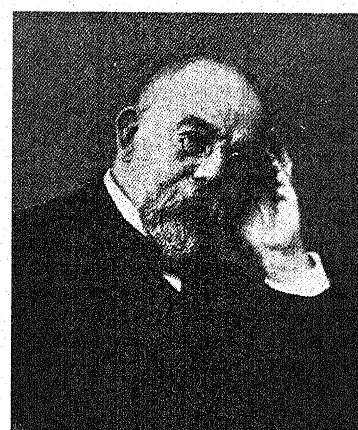


Fig. 9.—Robert Koch (1843–1910). (*Courtesy of Captain Henry J. Nichols, U. S. Army.*)

and the recognition by Pasteur that putrefaction, fermentation, and decay are due to micro-organisms which have parents as do other plants and animals. A third great advancement was made in 1876 when Weigert used some of the aniline dyes to stain the body of bacteria, thus making it possible to see and study the shape and structure of organisms which may occur in body tissue, soil, milk, and water. Still a fourth milestone on the path of bacteriological research was passed when the immortal Robert Koch devised the gelatin-plate method for obtaining pure cultures of bacteria. He added to liquefied portions of sterile gelatin small quantities of the material containing

the bacteria which he wished to study, shook the mixture to distribute the organisms uniformly throughout the liquid gelatin, and spread it on a covered sterile plate. The bacteria were fixed in isolated spots where they multiplied until they had formed colonies large enough to become visible to the naked eye. These colonies, each the offspring of a single cell, could furnish only one kind of bacteria. These could be fished out with a sterile platinum needle, studied under the microscope, grown in different media, perchance in soil, or the body of sus-

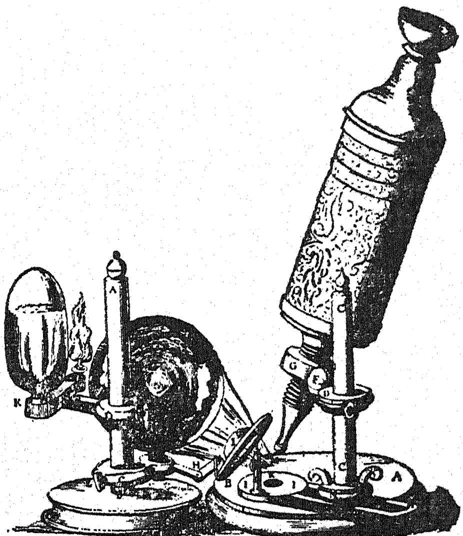


Fig. 10.—Hooke's compound microscope. (*After Gustave Fassin, Scientific Monthly.*)

ceptible animals until their shape, function, and various other characteristics were understood.

Many writers take 1882, the year in which solid media were first used, as the birth of the new science bacteriology. The reason for this becomes clear when we review the methods used up to this time in obtaining pure cultures of micro-organisms.

Pasteur, in his wonderful observations on yeast, obtained his pure cultures of yeast by feeding these organisms on a food especially suited to their needs. In this they grew rapidly and in time crowded out those less suited to the medium. This was

a long and tedious process and could be successfully used only where organisms had great physiologic differences.

Klebs (1873) tried to obtain pure cultures by a fractional method. He simply removed those organisms which grew most luxuriantly to another flask, making special cultures of these and so on until comparatively pure cultures were obtained. Lister (1878) and Nägeli (1879) obtained pure cultures by

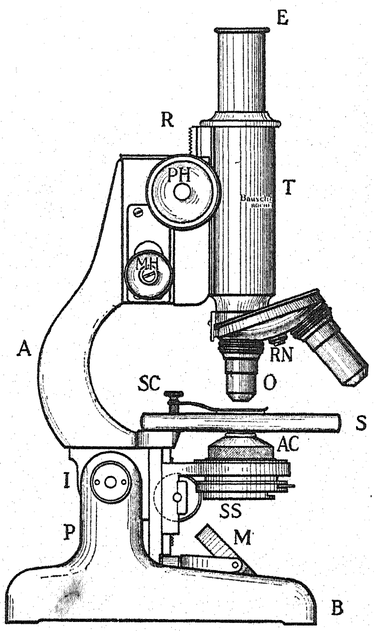


Fig. 11.—Student's modern microscope. *A,* Arm; *B,* base; *E,* eyepiece; *I,* inclination joint; *M,* mirror; *O,* objectives; *P,* pillar; *R,* rack; *S,* stage; *T,* body tube; *AC,* Abbé condenser; *SC,* spring clip; *SS,* substage; *RN,* revolving nosepiece; *MH,* micrometer head; *PH,* pinion head. (*Courtesy Bausch and Lomb.*)

diluting and transferring to special flasks decreasing amounts of the medium. Theoretically, if the dilution is carried far enough some flasks will receive only one organism from which there will develop a pure culture. But it can readily be seen that such methods depend on chance, and chance is against the worker where two organisms develop in close association with each other.

Prior to this, Roberts (1874) and Cohn (1876) had obtained pure cultures of some spore-bearing bacteria by subjecting the medium to a temperature of 100° C. at which all the nonspore-bearing forms were killed. This method was very limited in application; hence, it was not until the introduction of the solid cultural method by Koch (referred to above) that a scientific method of studying bacteria became available.

CHAPTER III

THE CONQUEST OF DISEASE

MAN has lived on this planet for thousands of years, yet until the middle of the eighteenth century he had not subdued a single disease. Since that time he has worked marvels. Many diseases have been controlled—some completely conquered. Portions of the earth's surface which have been stripped of animal life and made barren by disease have been restocked and made a fit place for the production of human food. Regions which had been seething hotbeds of pestilence have been converted into the most healthful spots on earth. Since the birth of bacteriology this world has been made a more decent home for man to live in, love in, marry in, rear his children in, and to die in at a riper age; for during this time man's natural span of life has been doubled.

Smallpox.—If humanity of the past could march in military array before the eyes of humanity of today arranged in armies according to the cause of their demise, what a spectacle it would be! One of the greatest armies would be composed of the victims of smallpox. Foremost among its ranks would be the countless billions of prehistoric cave men, followed closely by the millions from India. There would be divisions on divisions of Chinese from the pre-Christian era to the nineteenth century. During the short period of twenty-five years no less than 15,000,000 Russians joined the ranks; yes, probably every seventh child born in Russia for centuries would be found in this army. Sixty million men, women, and children joined it during the eighteenth century, whereas during the same period only 19,000,000 joined the army who lost their lives on the battlefield. Since the dawn of history millions from Europe, Asia, and Africa have joined, each century. At the beginning of the sixteenth century we should find millions from the new world marching hand in hand with those of the old world. In the ranks we should see the savage and the civilized, the ignorant and the educated, the strong and the weak, the old and the young, the prince and the pauper, the king and the queen, and

as we review the procession we should indeed conclude that no one was safe from the ravages of smallpox. We need only turn a few pages in the annals of history to understand the terrible devastation, havoc, and dread that this disease has caused among mankind since the beginning of time. "It granted no favor to old, it smote the middle-aged, it struck down the young, it scarred the unborn babe. None were so lowly as to be passed by without notice, none so powerful as to enjoy immunity. Not only did it stalk through the narrow alleys, but it walked abroad on the boulevard. It lay on the toiler's cot of straw and parted the purple curtains of the emperor's bedstead. Long before socialism was heard of, smallpox proclaimed 'special privileges to none.'" It is no wonder that "in the course of ages, the human race accumulated such fear of the eruptive fever that when the epidemic swooped upon the crowded communities of civilization there were times when sick infants cried in vain for mothers who had fled. When its pimpled visage appeared in the untamed forest an Indian father would call his family together, speak to them of the evil spirit which was torturing the tribe, and pointing to the dehumanized features of those already attacked would exhort his children to escape a similar fate by falling upon their own daggers, promising them if they lacked the courage that he himself as a last proof of his devotion would do the deed of mercy and at once follow them to the happy land." Truly, in those days, no mother could count her children until they had had smallpox for "from smallpox and love few remained free."

Continuing to view our visionary army we note at the close of the nineteenth century a marked difference in the number and kind of individuals who march in the files and ranks of the smallpox army. It no longer contains one tenth of the human race, but at times only a few stragglers. It is no longer made up of all sorts and conditions of peoples, but there appears to have been a selecting action. The doctors and nurses who so often helped to swell the ranks in the previous centuries have disappeared, and the ranks are made up more of the ignorant and less progressive. Some nationalities, as the English, Irish, Scotch, German, Swedish, Philippine, and Porto Rican, have nearly disappeared from the ranks, whereas the Belgian, Russian, Austrian, Spanish, and American races are there in greater numbers. Why this change in number and in nationality?

This question carries us back to the last quarter of the eighteenth century, when in the office of a country doctor a young girl made the chance remark, "I cannot take that disease (smallpox) for I have had cowpox." These words found fertile soil in the mind of young Jenner who, as he went about his work, pondered over the statement and wondered if there could be any truth in the strange belief of this country girl. He not only pondered over the subject, but he sought out and listened to the gossip of the cowherders and milkmaids as if he were collecting the folklore of a disappearing race. He learned to tell the true form of cowpox from the spurious types. He gleaned all the

Fig. 12.—Edward Jenner (1749–1823). (*From the painting by Sir Thomas Lawrence.*)

information on the subject that it was possible for him to obtain. He even persisted in talking of it at the meetings of a medical society to which he belonged until the members became bored and threatened his expulsion if he persisted. After twenty-five years of study and meditation the opportunity presented itself for a test of this perplexing problem. A dairymaid, Sarah Nelmes, had been pricked by a thorn and while milking a cow had become infected with cowpox. On May 14, 1796 Jenner took matter from her hand and placed it in a small scratch on the arm of an eight-year-old boy, James Phipps. Six weeks later the boy was inoculated with smallpox, a method that was universally used at that time to protect against it. The boy

showed no ill effect from the inoculation. This test was repeated in various places by different individuals, always with the same results. Jenner published his findings in a small 75-page book. In it is an engraving of the hand of Sarah Nelmes—"the hand that helped to halt a plague."

After considerable opposition from the haters of progress, vaccination spread over the world. Civilized and uncivilized, the great and the lowly, availed themselves of the great protective factor. Jenner lived to see that disease controlled of which it has been said, "In those stricken days, if a messenger had come from heaven and standing on earth's highest hill had clarioned to all mortals: 'From the long rôle of evils I shall remove one disease; which shall it be?,' one universal voice would have ascended in answer, the desire of kings blending with the prayer of peasants, the cultured accents of the scholar mingling with the cry of the man of the streets: 'Smallpox.'" In those nations in which vaccination has been made compulsory, smallpox has disappeared. In other nations it is still prevalent, and as the inhabitants forget the horrors of the past they neglect or oppose vaccination until reminded by the appearance of the grim destroyer. No one who has read the history of the past and the present has the temerity to claim that vaccination does not protect against smallpox. Yet some do say that there is danger in vaccination. To those, it can be said without fear of contradiction that the one who submits to vaccination is not endangering life nor limb and that the fear of vaccination is uncalled for.

The change in the number and personnel of the smallpox victims has come about through the battle of a country doctor against the king of diseases with "a little virus on the point of an ivory lance." So great was his victory that if every one were vaccinated and revaccinated, as medical experience shows to be necessary, the recruits to the smallpox army would absolutely cease, and in a few generations the horrible scourge (smallpox) would be a historical curiosity.

Pébrine.—Pébrine, a disease of the silkworm and named from black pepper-like spots appearing in the body of the infected silkworms, was one of the first diseases successfully controlled by Pasteur. He commenced work in 1865 "when the silk industry had been going from bad to worse. France, Italy, Spain, Greece, Turkey, China were all involved; the loss and the misery, down in the south of France, were terrible: It needed only

a few more turns of the screw to squeeze the life out of this great national industry." It was undertaken by Pasteur as a patriotic duty at the request of an old friend, Dumas, one who admired, had confidence in, and could foresee the greatness of Pasteur. For five years Pasteur struggled on without sufficient equipment, facing criticism and discouragement, suffering the loss of a kind father, of two loving children, and he himself brought near the brink of the grave by a cerebral hemorrhage which left him a cripple. In spite of these obstacles, in the end Pasteur won. He believed that the black spots in the body of the diseased silkworms were disease-producing micro-organisms. After long and careful study he proved that the disease is not

Fig. 13.—Louis Pasteur (1822–1895).

only communicable but maternally transmitted. He found that diseased moths lay diseased eggs from which developed diseased silkworms. These soon died or were useless in the production of silk. The germs left on leaves or in dust soon perished, but the ones in the eggs survived. Pasteur proved that the disease might be ended by the destruction of all diseased eggs. To this end he devised the process of placing each female moth when ready to lay its eggs on a separate piece of linen. After it had laid its eggs and adhered to the linen it was dried, removed from the linen, ground up in water, and the water examined microscopically. "If 'corpuscles' are found in it, the whole of the eggs of this moth and the linen on which they were laid are burnt; if no 'corpuscles' are found, the eggs are kept

to be hatched in due time and yield healthy silkworms." When the method was announced the cry came back: "It is impractical." To this Pasteur impatiently answered, "Don't tell me that anyone wants anything simpler than a preventive method which is just to put your eye to the eyepiece of a microscope after pounding a moth in a mortar with a few drops of water—mere child's play, taking an hour or two to learn." But the prejudice, falsehood, and bickering were overcome only by Pasteur personally taking over an old, badly infected, valueless (in so far as the production of silk was concerned) plantation and transforming it into a profitable, productive disease-free establishment. By so doing he demonstrated the practicability of his method, rescued the silk industry, and saved for France something like four millions of dollars yearly.

Anthrax.—The second disease to be conquered was anthrax, a disease highly fatal and widely prevalent among the lower animals. With man it is quite rare and less fatal. It is referred to in both sacred and profane history, modern and ancient. No one knows when or where it had its origin. Until the last quarter of the eighteenth century man stood in helpless astonishment before its fatal and rapid action. Its causative organism was the first pathogen to be seen by man, the first to be obtained in pure culture, and one of the first to be conquered. The organism was seen and described by Pollender in 1855, but he failed to prove that it was the cause of anthrax. Davaine, in 1863, all but reached the coveted goal which was attained by the young German bacteriologist, Robert Koch, in 1876. Koch obtained the organism in pure culture and proved it to be the specific cause of splenic fever (anthrax).

In 1877 Pasteur set out to discover a cure or a prevention for anthrax. For years he struggled on. He traveled through infected districts, listened to the farmers' stories, studied the disease's natural history, and discovered the organism causing it in the body of earthworms which had fed on the carcasses of animals dying from anthrax (thus, showing how it may be carried about in the soil). He grew the micro-organism in test tubes, and proved that it could be transferred from flask to flask and still produce the disease in susceptible animals. He cultured it at abnormal temperatures, 42° C., injected it into the bodies of animals and found that they did not contract the disease. Such cultures rendered the animals immune, that is, the disease could not be caused in animals subjected to this treat-

ment. After repeated verification of the results, and after most careful analysis, he announced to the world that he had found a prevention for anthrax. His announcement fell on the ears of scoffers and skeptics. One veterinary journal in 1881 carried the following: "Will you have some microbe? There is some everywhere. Microbiolatry is the fashion; it reigns undisputed; it is a doctrine which must not even be discussed, especially when its Pontiff, the learned M. Pasteur, has pronounced the sacramental words, 'I have spoken.'" Ridicule, sarcasm, and even personal abuse was not to deter the man who had fought through sorrow and illness and who knew that he had discovered a great truth. Great, indeed, was the astonishment and surprise when Pasteur announced that he would submit the question to a decisive public test. Fifty sheep were to be given the virulent anthrax bacteria. One half of them were to have been previously protected by the vaccine, while the other half were not. Pasteur predicted that the 25 unprotected sheep would perish, while the protected ones would survive. On May 5th the animals to be protected received their first dose of the vaccine. Twelve days later they were given a stronger dose. On May 31st both groups were given the virulent anthrax organisms. On the afternoon of June 2nd the crowd, composed of farmers, veterinarians, physicians, journalists, and scientists from near and far, met to learn whether Pasteur was a dreamer or a prophet. What they saw was one of the most spectacular scenes in the history of peaceful science, a scene which Pasteur afterward declared "amazed the assembly." Scattered about the enclosure, dead or dying, were the unprotected sheep. Twenty-two were dead; the other three survived only a short time. The vaccinated sheep were in perfect health!

The results created a tremendous excitement throughout France. It was not only a victory for Pasteur, but it also meant a great saving to his noble countrymen, and it placed the name of his beloved war-crushed France one notch higher in the hall of fame. To mankind it marked a victory of far-reaching importance in the conflict which man is waging against disease.

Rabies.—In the beginning rabies, sometimes called hydrophobia, was confined to the lower animals. As man domesticated animals he shared their diseases. It is therefore likely that even during the childhood of the race the cry of the mad dog struck terror to the heart of man. This great fear of the disease has come from two causes: First, the mortality which is

practically 100 per cent; and second, there are few, if any, other afflictions in which the suffering is so intense. So great is this that no sane man would witness it a second time were he not needed to aid the unfortunate victim. Pasteur sensed these facts, and oh, how he longed to bring relief! He was now past the prime of life, an invalid, and had written his name securely in the history of science. Many a less ambitious man would have rested on his laurels and enjoyed, as the world sees enjoyment, his declining years. Not so with the maker of a new science, for in December, 1880 he entered upon the arduous self-imposed task of love: To find a preventive, or a cure for the awful malady. Not only was the work difficult, but it was beset with grave personal danger. Pasteur did not fear the danger, and he hoped to overcome the difficulties. The causative organism was unknown. All that was known was that the disease was conveyed by the bite of a rabid animal.

Pasteur firmly believed it to be caused by a micro-organism. He had helped to overthrow the idea of spontaneous generation and prove that every micro-organism is the offspring of a similar one; hence he reasoned the virus causing this disease should be found in the mouth of the afflicted animal. The saliva of the diseased child and dog was inoculated into susceptible animals. The results were various. At times the animal died of rabies, but more often it died of septicemia (blood poison). The blood was tested with no better results. Pasteur reasoned: "Inasmuch as rabies is a disease of the nervous system, the cause should be sought in the nerves." Parts of the spinal cord of animals dead of the disease were grafted under the skin of the rabbit. The results were indefinite. They were placed on the brain of experimental animals and rabies invariably resulted, but the latent period (the time between the introduction of the material and the appearance of the symptoms) varied. How could the latent period be made constant? The spinal cord of one rabbit dead of the disease was used on a second, and this on a third, and so on. The latent period grew shorter and shorter, until in time the rabbits always died between the sixth and seventh days. Pasteur had obtained what he called "fixed virus" in distinction to the ordinary or "street virus."

After numerous and varied experiments, he found that the spinal cord of an animal dead of the fixed virus could be dried under appropriate conditions and used to immunize animals against rabies. The treatment consisted of giving the animal

as soon after being bitten as practicable a salt solution of the dried cord and later on another solution of cord which had been dried for a shorter period. In time, the experimental animal could receive the undried diseased cord without ill effects. The animal had become immune. In principle, it is as if an individual were to receive two months hence a fatal dose of morphine. He is given today and each day thereafter small but increasing doses of the drug. When the date for receiving the fatal dose is reached, he takes it with impunity.

The treatment was tried on thousands of animals which had been bitten at varying times, under different conditions, and also on animals which had been given the nervous tissue of rabid animals. The results were always the same. When given just before or soon after the infection the animal never developed rabies. Still Pasteur hesitated to try it on man until circumstances crowded it upon him. The occasion soon offered itself.

On Monday morning, July 6, 1885, an Alsatian woman brought her child to Pasteur's laboratory. The boy had been attacked while on his way to school by a mad dog. He had been knocked down and bitten in fourteen places. When rescued by a bricklayer his wounds were found to be deep and covered with the saliva of the rabid animal. He was taken to the village doctor who carbolized his wounds and sent him to Pasteur who examined the boy and was greatly touched by his suffering and prospects, for wounds such as he had received invariably proved fatal. Pasteur hesitated to give him the treatment. The mother insisted. Pasteur's advisers intimated that the boy's death would be upon Pasteur if he refused to treat him, and the mother absolved him from all responsibility if the treatment were given. Against the advice of friends Pasteur began the treatment upon the boy, shortening the time between the treatments in an effort to secure a cumulative effect. The child in perfect confidence ate well, slept soundly at night, and played about the room during the day. Pasteur spent sleepless nights and anxious days watching for the least ill symptom which might appear. The crisis passed. Pasteur had conquered hydrophobia! In October he saved his second patient, the young shepherd Jupille, who to protect some small boys had collared and killed a mad dog. In so doing he was frightfully bitten. Six days later he received the treatment and was saved.

The news of these 2 cases rapidly spread and brought a rush

of patients to Pasteur. Soon Pasteur institutes appeared in available centers throughout the world, and today the mortality of the malady which formerly was nearly 100 per cent has been reduced to less than 1 per cent.

Pasteur Honored.—Pasteur had been the subject of criticism and distrust. His discoveries had been received with skepticism and forebodings of evil. He had struggled on at times almost alone, amidst poverty, sickness, and sorrow with the sincere desire to help his beloved France and especially to alleviate the ills of man. He lived to realize many of his hopes and to be the most honored man of his age. He was the recipient of honors from learned societies. Scholars paused to pay him tribute. Even the laity recognized his greatness, as was shown by the great popular vote that was taken in which Pasteur was acclaimed the greatest man of his age winning over such heroes as Napoleon and Victor Hugo. When subscriptions were called for to erect Pasteur institutes in which his work could be continued they flowed from all quarters of the globe—the offerings of the rich and the poor, the great and the lowly. On his seventieth birthday delegates from scientific societies and public bodies met in France to do him honor. The President of the Republic escorted Pasteur to the rostrum where he was lauded as one of the greatest heroes and benefactors in human history. Three years later when he died his remains were laid away amidst the tributes and sorrows of a grateful world in a magnificent chapel. In the vault over his grave stand erected four great white angels—Faith, Hope, Charity, and Science.

Yellow Fever.—For centuries some of the most fertile areas of the New World were uninhabitable to the white race, or if perchance any of this race settled in those regions the appearance of yellow fever forcefully reminded them that they must move or die. Ninety times it invaded the United States, often enacting scenes which were the counterpart of the plague days in London.

For four centuries the narrow Isthmus of Panama was regarded as the white man's grave. It is claimed that the first railroad constructed across it cost a human life for each tie. Ferdinand de Lesseps, who undertook to construct a canal across the Isthmus, was forced to abandon it, not through lack of engineering skill, but because he did not recognize the part played by the mosquito. His men died like flies. Common laborers, nurses, doctors, and engineers alike fell prey. One vessel

brought over from France 18 young engineers. Within a month after their arrival all but one had died. Before the work was finally abandoned thousands had lost their lives, and it was hard to keep enough well to care for the sick. Eighteen per cent of all the men employed had died, and many more were helpless. Twenty years later a canal was constructed and that with a mortality of slightly less than 16 per thousand! Today the mortality is less in the Canal Zone than in many of our large cities. The change is due to the discovery of the cause and method for control of the yellow fever.

Fig. 14.—Walter Reed (1851–1902).

As early as 1797 Dr. Rush of Philadelphia noticed excessive numbers of mosquitoes during yellow-fever epidemics. In 1848 Dr. Nott of Mobile, Alabama, suggested that yellow fever was disseminated by some insect "that remains very close to the ground." Five years later a French physician attempted to prevent the disease by disinfecting the bite. In 1881 Dr. Carlos Finlay formulated the mosquito theory of the transmission of yellow fever. During the rest of the century the hopes of man rose and fell with every theory that was promulgated or whenever a suspected organism was isolated only to be shown in the end that it was not the cause of the disease.

When General Wood was made military governor of Cuba in 1898 he immediately cleaned up the city Santiago and the surrounding provinces. He succeeded in making the city an exceptionally clean place, but his efforts were futile in so far as the eradication of yellow fever was concerned. Conditions became so bad that in 1900 General Nelson O. Miles had a commission appointed "for the purpose of pursuing scientific investigations with reference to the infectious diseases prevalent on the island of Cuba." The commission was composed of the bacteriologist, Walter Reed, and Drs. James Carroll, Jesse W. Lazear, and Aristide Agramonte.

They built two mosquito-proof houses. In one of these soldiers lived for a period of twenty-one days. By night they slept in bedding soiled with the vomit and excreta of sick and dying yellow-fever patients. This was repeated with three different detachments. Not one developed this or any other disease!

The second house contained two rooms separated by a screened partition. All of the furnishings which went with the house had been disinfected and were perfectly clean. Volunteers were placed in the two rooms. In the one mosquitoes were liberated which had fed on the blood of yellow-fever patients. All mosquitoes were excluded from the other: Fifty per cent of the men in the mosquito ward developed yellow fever and not one in the other ward, although they were separated only by a screen. On August 27, 1900 Dr. Carroll permitted an infected mosquito to bite him. His account of the experiment is as follows: "The insect, which had been hatched and reared in the laboratory, had been caused to feed upon 4 cases of yellow fever, two of them severe and two mild. The first patient, a severe case, was bitten twelve days; the second, third, and fourth patients had been bitten six, four, and two days previously, and their attacks were in character mild, severe, and mild, respectively. In writing to Dr. Reed on the night after the instance I remarked jokingly that if there were anything in the mosquito theory I should have a good dose; and so it happened after having slight premonitory symptoms for two days I was taken sick on August 31st and on September 1st I was carried to the yellow-fever camp. My life was in the balance for three days." Dr. Lazear had previously submitted to an experimental bite without effect; later he had allowed a mosquito, which settled on his hand while he was taking blood from a yellow-fever patient, to take its fill. After a short incubation period he de-

veloped the disease from which he soon died. Dr. Agramonte writes: "How can I describe the agony of suspense which racked our souls during those six days? It seemed to us as though a life was being offered in sacrifice for the thousands which it was to contribute in saving. Across the span of thirteen years the memory of the last moments come to me most vividly and thrilling, when the light of reason left his brain and shut out of his mind the torturing thought of the loving wife and daughter far away and of the unborn child who was to find itself fatherless on coming into the world. Tuesday, September 25th, saw the end of a life full of promise; one more name, that of Jesse W. Lazear, was graven upon the portals of immortality."

After further tests the following principles were established: Yellow fever is not contagious, nor is it conveyed by means of fomites. It is conveyed only by a species of mosquitoes (Aedes egypti) which, in order to be dangerous, must feed on the blood of a yellow-fever patient during the first three days of his illness, after which the insect is harmless to man for a period of twelve days. After this period its bite will convey the disease to the nonimmune person. Hence, to prevent the disease in man it is necessary to prevent the infection of the mosquitoes by man and to eradicate them by destroying their breeding places.

Between 1918 and 1924 Noguchi isolated on a number of occasions from the body of individuals ill of yellow fever a spirochete *Leptospira icteroides* which he came to consider as the cause of yellow fever, but inasmuch as Adrian Stokes and others have failed to reveal the leptospira but have obtained a virus that passes the closest grained pores of the finest filters the causative organism has come to be considered an ultramicroscopical virus. There has also been discovered a serum which, were it not for the fact that better preventive means are available, can be used effectively against the disease. But such work has been paid for by the lives of brilliant workers. Within recent years Adrian Stokes, Hediyo Noguchi and William A. Young have given their lives in trying to establish the nature of African yellow fever which has been one of the baffling problems of tropical pathology.

Other Plagues Conquered.—The discovery of antitoxin for diphtheria has reduced the mortality of this disease from 30 to less than 3 per cent, and the introduction of the antitoxin and toxin-antitoxin, or toxoid, foreshadows its absolute control. The work of the Dicks bespeaks a similar victory over scarlet

fever. Typhoid fever is all but controlled. Asiatic cholera and plague are fast being swept from the earth. Typhus fever and a number of other diseases are within the control of man. All this has come within three generations! What will the future bring?

Future Work.—One may think from the preceding that in this field of science there is little to be done, but this is not the case for there are diseases still unconquered. "Measles bends over every cradle; the bacillus that Koch saw in 1881 is as much a menace today as it was twenty-five centuries ago when Hippocrates called consumption the most dangerous disease; the swift and sudden onslaught of pneumonia carries desolation in its wide trail." Cancer is on the increase. Man stands helpless before the onrush of influenza. Pneumonia and meningitis demand their yearly toll. Other diseases claim their tens of thousands each year, while the physicians stand helplessly by and watch the losing fight of their patients. Surely, some future Pasteur will bring relief!

CHAPTER IV

MICRO-ORGANISMS BECOME THE ALLIES OF MAN

Bacteria at first were looked upon as curios which probably represented the origin of life; later, as the enemies of man; and still later it was learned that they are not only useful to man but absolutely essential to his existence. A few have become outlaws and prey upon life, but the overwhelming majority administer to the needs of life.

Fermentation.—Since the dawn of history man has been interested in the wonderful process known as fermentation, and although many an ingenious theory has been formulated to explain it, little more than theory existed until the classic work of Pasteur on fermentation appeared about 1857. Pasteur claimed that all forms of fermentation were due to the action of microscopical organized cells. An idea such as this, even at this late date, did not go unchallenged, for we find no less illustrious workers than Helmholtz and Liebig opposing it. The latter even scoffed at such an idea, writing: "Those who pretend to explain the putrefaction of animal substance by the presence of micro-organisms reason very much like a child who would explain the rapidity of the Rhine by attributing it to the violent motions imparted to it in the direction of Burgen by the numerous wheels of the mills of Venice."

Pasteur's early work was in chemistry. He prepared a double salt of racemic acid, a sodium ammonium racemate, and let it crystallize. These crystals were of two forms, one having the facet of the commercial acid and the other having the facet proper to the unknown acid. He separated the two forms of crystals, picking them out one by one. Solutions were prepared from each and tested with a polariscope. They were different. The one form rotated the plane of the polarized light to the right, the other turned the plane, through an equal extent, to the left. He had separated the crystals by hand; later he allowed micro-organisms to feed upon a solution of the two forms. One was used by the organism as food; the other was left untouched. And so Pasteur passed from the field of chemistry to

the realm of bacteriology. Due to this discovery there developed explanations of molecular structure and the use of micro-organisms for the separation and identification of closely related compounds.

Pasteur devoted much of his time for twenty years to a study of fermentation. During this time he obtained in pure cultures many micro-organisms and proved that each produced its own peculiar results or products of life activity. He showed that diseases of wine were due to foreign organisms and that heating to a comparatively low temperature made it possible to keep indefinitely products with their desirable flavors. Thus originated the modern process of pasteurization. His carefully planned and executed experiments which together with the logical conclusions drawn from them, put the brewing industries on a sound scientific basis, in place of the old cut-and-try method of the preceding periods. These discoveries alone saved France $20,000,000 yearly; and what is more important, they proved that without micro-organisms there would be no fermentation, no putrefaction, no decay of any kind except by the slow process of oxidation!

This was only one of the many processes studied by Pasteur. In 1857 he gave to the Lille Scientific Society his paper on lactic-acid fermentation. Four years later he discovered another fermentation which occurred after lactic fermentation and is known as butyric-acid fermentation. He noted the relationship between this and the rancidity of butter, and also demonstrated that the ferments grow only in the absence of air, thus establishing the fact that there may be life even in the absence of free oxygen. To these micro-organisms he later gave the name of anaerobes. In 1867 Pasteur taught the vinegar makers of Orleans how to speed up the fermentation of vinegar. The next year he taught France how to keep her rough wines from going sour. In 1871 he took a huge east-end brewery in London by surprise and showed them the impurities of their yeast and the evil effect it was having on the liquor. When driven from his laboratory to the home of his childhood during the siege of Paris he spent his time studying the fermentations in the tannery. "He would ask endless questions, trying to discover the scientific reason of every process and every routine. While his sister was making bread he would study the raising crust, the influence of air in the kneading of the dough, and, his imagination rising as usual from a minor point to the greatest problems,

he began to seek for the means of increasing the nutritive value of bread, and consequently of lowering its price."

The work of the Danish investigator Hansen in obtaining pure cultures of yeast is significant. He succeeded in seeding cultures with a single cell and separating one species from another. He proposed methods for the differentiation of species, worked out their life cycles, and proposed a classification which in recent years has been universally accepted.

Fermentation and Breadmaking.—The importance of bread to mankind is voiced in the supplication: "Give us this day our daily bread." Wells states: "Secondary foods may be more important than bread with certain classes of society—the rich, for example—in certain localities, and at certain limited periods; but throughout civilization and beyond, bread and bread alone is the basic common food. What milk is to the infant, bread is to the world." The nature and composition of bread varies greatly in different localities and with different people, but in all cases it is the commonest food and is made of the commonest cereals of the land. Its biological interest comes from the methods used in leavening, the spoilage due to micro-organisms, and the likelihood of carrying human pathogens.

We do not know when fermentation first entered the baking industry. Probably before the dawn of modern history man discovered that crushed grains mixed with water and kept in a warm place undergo a desirable change, which today is known as fermentation. Leavened bread was used in the days of the ancient Israelites, for we are told how in the flight of the Israelites out of Egypt "the people took their dough before it was leavened, their kneading troughs being bound up in their clothes." The art of leavening passed from the Egyptians to the Greeks, and from the Greeks to the Romans, whose conquests and colonies extended the art. At first it consisted of a spontaneous fermentation of the dough, later, it became the custom to hasten by adding a portion of old fermented paste or "leaven." Still later, yeast was substituted for the piece of leaven, and the process of breadmaking passed from the realm of chance to that of a science. From start to finish, breadmaking is a biological process, and, as pointed out by Wells, it is the use of a leavening agent that has transformed wheat from the poorest to the best bread grain.

"Since he first found out how to use fire to cook his food, man has made but two other really important food discoveries.

These are the preserving of meats and other perishables by salt, smoking, or drying, and the use of leavening in breadmaking. These two discoveries are both prehistoric. The importance of leaven in breadmaking cannot be over-estimated, because without leaven, wheat and rye would never become leading food grains. Without leaven the cultivation of wheat and rye would now cease. Without leaven wheat is the most stubborn and intractable of possible foods. Even an amateur cook can make an edible bread from Indian corn, or barley flour and water without leaven, but a professional would be stumped to make anything edible from wheat flour and water alone. But with leaven, yeast, or baking powder, wheat becomes the supreme bread-making grain. In other words, wheat, the least suitable of all grains for use as

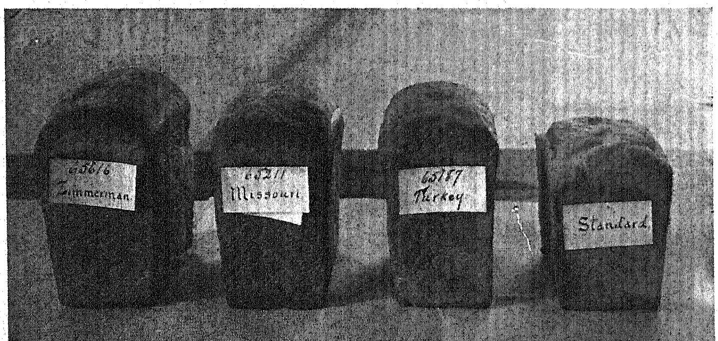


Fig. 15.—Bread made from flour of different gluten content.

a human food, with leaven becomes the most suited. This is because it is best suited for making that kind of food which is the base food of all civilized peoples. Here we are at the root of one of the most significant facts of present day civilization. Man by the discovery of leaven has been able to raise one grain, and that in its natural state, the least promising of all grains, to be not only the prime grain but the prime food as well."

At first the leavening agent was that which chanced to adhere to the grain; later it became the custom to add to new dough a bit of old fermented paste or "leaven," and still more recently yeast was substituted for the piece of leaven.

Some believe that the main function of the yeast is to aerate the bread and the famous German chemist, Liebig, at one time advocated chemical aerating agents. He laid great stress on the

loss of nutrients due to the use of micro-organisms. But we now know that profound changes are wrought by the micro-organisms on the carbohydrates and especially the proteins of the flour, and the lightness, sweetness, and digestibility of yeast-made bread far surpass that made with chemical aerators.

The leavens used in breadmaking may be roughly divided into three classes.

1. The comparatively pure yeast obtained from distillers' wort and which is placed on the market as compressed yeast, dried yeast, and liquid yeast.

Compressed yeast is collected from the wort, thoroughly washed, passed through wire and silk sieves in order to free it from foreign material, and pressed. It is then cut into cakes and wrapped in tin foil. This form of yeast keeps but a short time and spoils quickly; so it must be kept cold. Dried yeast is made by mixing fresh liquid yeast with cornmeal, flour, or other starchy materials, compressed into cakes, and dried at a carefully regulated temperature. If properly made this yeast keeps for a considerable time. The percentage of living cells in dried yeast is small as compared with the compressed yeast, and the numbers gradually decrease with time. Liquid yeast is collected directly from the distillers' wort and dispensed as a concentrated liquid. It keeps poorly and is likely to be contaminated with bacteria.

2. The yeasts propagated in the bakery, or the home. These are made by growing yeast on media made of potatoes, sugar, hops, and various other constituents.

3. The spontaneous leaven which develops in mixtures of flour and water when kept under appropriate conditions. These are mixtures of yeast and bacteria which occur on the grain. Comparatively large quantities of salt are often added to prevent the growth of the high acid-producing bacteria.

Biological Changes During Leavening.—Carbon dioxide is evolved which gives porosity to the bread. Some of the starches and proteins are split and are then more easily assimilated by the body. The gluten is softened and certain characteristic flavors and aromas are developed.

Abnormal Fermentations of Bread.—The most common abnormal fermentations in bread are ropiness, undesirable high acidity, and "bloody" bread. Ropiness, or sliminess in bread results after baking from the growth in the bread of certain slime-forming highly resistant spores. These may have come from

the yeast, the flour, or the various utensils used in the bread-making process, and when they get into the bakery become a real nuisance. The acid developed in normal fermentation tends to prevent their growth and at times acid is added. Sour bread sometimes results from excessive growth of lactic and butyric acid bacteria. They may reach the dough in excessive numbers from the flour, water, and impure yeast. They give to bread bad flavors. Large quantities of good yeast tend to keep them in check. Colored spots may occur in bread due to the growth within it of chromogenic bacteria. One of the most common is *Serratia marcescens* which causes the so-called "bloody" bread.

Bread and Diseases.—Unwrapped bread if carelessly handled may become infected and convey human infection in this manner; but the modern automatic bakery machinery along with the automatic wrapping machine makes it possible for the baker to guarantee the public bread free from disease germs. Consequently, today many bakers truthfully advertise "bread which has never been touched by human hands." Such bread would not convey pathogens.

Soil Bacteriology.—It was a well-accepted maxim with the older writers of agriculture that "corruption is the mother of vegetation." One of the most illustrious of their number, Liebig (1855), taught that plant and animal tissues decayed in the soil with the formation of ammonia which he considered highly essential to plant growth. Part of this ammonia according to his belief was transformed into nitric acid which also served as plant food.

Long before the nature of the process by which plant residues and manures are transformed into nitrates was understood the method was used to supply the large quantities of gunpowder consumed in the almost incessant wars of Europe. In the eighteenth century the artificial production of saltpeter in beds of decaying organic matter reached a high degree of perfection. Especially was this true in Sweden, Switzerland, and France where nitrates were collected as a part of each farmer's tax. In the year 1777 the French Government issued special instructions for its manufacture in which there was stressed the form of pit to use, the covering of organic matter, the arrangement for the free entry of air, the optimum amount of moisture, and the necessity of a base to neutralize the acid produced.

Boussingault, a French chemist, in the years between 1860 and 1878 became interested in the natural-occurring "niter

beds," especially those of Peru and Ecuador. He did work which established the origin of the nitrate as coming from the organic matter of the soil. It was considered, however, that the transformation was a chemical change produced through the interaction of ozone or active oxygen on the ammonia coming from the organic matter. The soil was supposed to act as a catalyzer, thus accelerating the speed of the reaction.

During the sixties and seventies great advances had been made in bacteriology. It had been definitely established that bacteria cause decay, putrefaction, and fermentation, and it was conceivable that they were the active agents in changing the ammonia within the soil. Pasteur had expressed the opinion that nitrification, the changing of ammonia into nitrates, was a bacterial process, but nothing definite was known on the subject until 1877, when Schlösing and Müntz were surprised by the peculiar action of sand filters used in purifying sewage.

A continuous stream of sewage was allowed to trickle down a column of sand and limestone at a rate such that it required eight days to pass. The first twenty-one days the ammonia in the sewage was unaffected. Then it began to appear as nitrates, and in time only nitrates issued from the filter. If it were a chemical process, nitrates should be found at once. Why must twenty-one days elapse before the ammonia was transformed into nitrates? In attempting to answer this, they found that the process could be stopped by the addition of chloroform and started again by adding the extract of fresh soil. Nitrification was thus shown, to use their expression, to be "due to organized ferments."

The succeeding thirteen years represent the history of a race among the leading scientists of Europe and America to determine to whom should go the honor of first obtaining and studying in pure culture the micro-organisms which possessed this important property of transforming ammonia into nitrates.

Warrington of England proved that no matter what compounds of nitrogen are supplied to a plant as manure they are rapidly transformed within the soil into nitrates. Furthermore, he found that ammonia is first changed to nitrite and then into nitrate.

However, up to the time Winogradsky took up the subject, all attempts to isolate such organisms had failed. After long, trying experiments which demonstrate the keen, untiring ingenious nature of this investigator, he gave to the world nitrifying organisms. He found they would not grow in the ordinary media

that had been used by previous workers; the media must be free from organic matter.

It has been known for generations that uncropped soil increases in fertility and that legumes differ from other plants in their nutritive requirements. Less ancient, however, is the knowledge that uncropped soil may gain in nitrogen or that legumes with the aid of bacteria may get their nitrogen from the air.

In the middle of the nineteenth century Boussingault wrote: "Vegetable earth contains living organisms—germs—the vitality of which is suspended by drying and reestablished under favorable conditions as to moisture and temperature." He also hinted

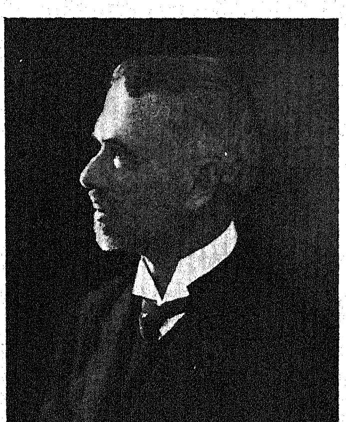


Fig. 16.—S. Winogradsky. (*From Waksman's, Principles of Soil Microbiology. Williams and Wilkins.*)

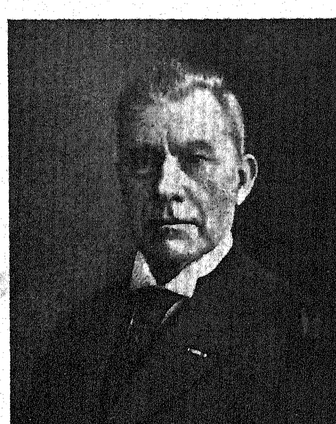


Fig. 17.—M. W. Beijerinck (1851–1931). (*From Waksman's, Principles of Soil Microbiology. Williams and Wilkins.*)

at the fact that these micro-organisms take nitrogen from the air and change it so that it can be used by the growing plant. He spread out thinly 120 Gm. of soil in a shallow glass dish and for three months moistened it daily with water free from nitrogen compounds. At the end of this time he found that it had gained nitrogen but had lost carbon. Thirty years later Hellriegel and Wilfarth solved the apparently hopeless problem of the nitrogen nutrition of leguminous plants. They found that bacteria live in little nodules on the roots of legumes and manufacture nitrogen compounds which they give to the higher plants in exchange for carbohydrates and other nutrients needed by

bacteria. Since then we have learned much concerning the relationship of plants to free and combined nitrogen of the air and the soil.

In 1901 Beijerinck discovered large yeastlike micro-organisms which live free in the soil and take from the atmosphere nitrogen and build it up into complex compounds within their bodies. Every year since this date the number of micro-organisms which have been found to possess this property has been added to, until today many micro-organisms possessing this important property are known.

Terrific battles are being waged in the soil between the microscopical plants and microscopical animals. This was the conclusion reached by Russell and Hutchinson in 1909. Since that date each year has added new knowledge concerning organisms already known or else added new ones to the list of beneficial organisms, until we now know that the soil is teeming with organisms most of which are beneficial. Some of them, however, are without significance, and a few of them are injurious. Micro-organisms not only deal with nitrogen and organic matter of the soil but they act on sulphur forming sulphuric acid; they form acids which liberate phosphorus, potassium, calcium, and other minerals needed by the plants. It is the problem of the worker in soil bacteriology to learn how to speed up the work of the beneficial and to suppress or weed out the injurious. Advances have been made in this realm, but for the most part it is a virgin field which awaits the coming of the untiring worker who cares to thrust in his sickle and reap. For, truly, the harvest will be rich enough to satisfy the most fastidious.

CHAPTER V

BACTERIA: OCCURRENCE AND FUNCTION

BACTERIA rule the world. Man is dependent upon them from the day of his birth until the hour of his death, and even when that hour comes they are present. They turn out the light and take possession of the body. They are man's most useful servants and his most destructive master. This being true, one is prone to ask: What are bacteria? Where do they occur? What are their functions?

What Are Bacteria?—Bacteria are very minute single-celled living beings devoid of roots, leaves, and stems. They are so small that they can be seen only with the aid of a powerful microscope; consequently, they are often spoken of as microorganisms. This term includes not only bacteria but all forms of life so small as to require the microscope in their study. They are often referred to in common usage as germs or microbes. The early investigators considered them animals; hence, they referred to them as "animalcules." The terms bacteria, germs, or microbes usually suggest to the layman a minute animal, and we often hear them spoken of as "bugs" even today. On examination we find that bacteria have many of the characteristics of animals. Some have the power of independent motion. All are devoid of green coloring matter, chlorophyll; hence, most of them are compelled to live upon complex foods as do the animals. Their general structure, their methods of growth, their formation of threads and spores, and their similarity in general to some of the lower forms of plant life, such as the green algae, have caused the biologist to class them as plants. However, it is impossible to make a clear-cut distinction between some microscopical plants and some microscopical animals. This is not surprising, for long before these simple forms of life were known the terms, "plants and animals," were invented to distinguish familiar living objects, such as elephants and trees, insects and mosses. Haeckel suggested that to obviate this difficulty we consider bacteria neither as plants nor as animals, but that we create a new kingdom to be known as the Protista,

into which we could place all of the lower forms of life. However, this does not solve the problem, for we would then have to draw two lines of demarcation in place of one. The important thing to remember is that bacteria are at the very foot of the ladder—the simplest forms of life, and hence, partake of the characteristics of both plants and animals. For this reason and for convenience, scientists have agreed to consider the bacteria with the plants.

The plant kingdom consists of four great divisions: (1) The seed plants or Spermatophytes, (2) fern plants or Pteridophytes, (3) mosses or Bryophytes, and (4) thallus plants or Thallophytes. The Thallophytes have neither roots, stems, nor leaves. There are two main divisions of the Thallophytes: (A) The algae which possess the green pigment chlorophyll and are, therefore, able to manufacture their own food from the carbon dioxide of the air; and (B) the fungi which lack chlorophyll and, consequently, are obliged to obtain their food from living plants or animals or from dead organic materials. To this division belongs the great array of bacteria, molds, yeasts, blights, rusts, toadstools, mushrooms, and similar plants.

Nor is it an easy task to differentiate nicely between bacteria, yeasts, and molds. They may roughly be distinguished from each other as follows: Bacteria are single-celled plants containing nuclear material which is intimately diffused throughout the cell. They multiply by transverse fission—a pinching in two at the center of the organism. Sometimes they are united into long chains which are easily separated into their individual links. Each link is a complete organism. Yeasts are usually larger than bacteria. They are single-celled organisms and have a definitely organized nucleus. This sets them off from bacteria. They multiply by budding, that is by the formation of buds, or small protuberances, which appear on the side of organisms, enlarge, and two daughter cells are formed. Molds consist of numerous cells. These are a mass of interwoven threads, each thread being composed of a number of cells which function as a unit.

Where Do Bacteria Occur?—Bacteria are widely distributed, occurring nearly everywhere. They are found in all soils, the number varying with the kind of soil, quantity of plant and animal débris present, moisture, and treatment. They decrease in number with depth, but many soils of Western America contain numerous micro-organisms even at a depth of 10 feet. Al-

though they occur in air, it is not their natural home, as under ordinary conditions they cannot grow and multiply in it. The number and variety found in air vary with a number of factors—location, moisture, dust, movement of the atmosphere, and the presence or absence of injurious gases. The atmosphere of some high mountains or glaciers and the air over the ocean far from shore is practically free from bacteria. City and country air also differ from each other in the number and kind of bacteria which they contain. There is a great variation in the air of buildings. They are especially numerous where dust is plentiful.

Most natural waters contain great numbers of bacteria. In sewage and polluted water they are especially numerous. They occur only in small numbers or not at all in deep wells and springs; shallow wells, ponds, and streams draining inhabited districts often contain as many as millions in 1 cc. A turbid stream, like the Mississippi which contains the drainage of many cities, has a great variety and number of bacteria in opposition to the clear, rapid flowing water of uninhabited mountainous regions. Great Salt Lake, with its large quantities of common salt, contains few micro-organisms, whereas the Great Lakes in the central part of the United States contain many. According to Waksman: "The bacterial population of the sea is quite characteristic. It is distinct in nature from the population usually found on land, as shown by a more limited number of bacterial types found in the sea. Spore-forming bacteria which comprise an important part of the bacterial population in soil, are practically absent in sea-water, although they may be present in considerable abundance in the sea bottom. Cocci are also of limited occurrence in the sea. Motile rods and various types of vibrios, or comma-shaped organisms, usually make up the major part of the bacterial population thus far studied. The poverty of bacterial species in the sea depends largely upon the specific nature of sea-water as a medium for the growth of these organisms."

Milk, as secreted by the cow, is practically free from bacteria, but as it is drawn from the udder it always contains them. The number and kind vary with specific cows. Some healthy cows may give milk with as few as twenty bacteria in a cubic centimeter, whereas the milk of others may contain thousands, while that of diseased cows may contain millions. During the milking process milk receives germs from the coat of the cow, the clothing of the milker, the buckets, strainers, and other things with which the milk comes in contact. Often by the time it

reaches the consumer it contains millions of bacteria in every cubic centimeter.

Bacteria are found on the surface but not on the inside of undamaged fruit and vegetables. The normal tissues, sap, and heartwood of plants, are free from them. If injected into them the bacteria may survive for some time, but the tissues must die before they can serve as food for bacteria. All food, except that recently cooked, contains bacteria, the number and kind varying with the nature and age of the food.

Living as we do in a world filled with bacteria, we can expect to find them on the surface of the skin and mucous membranes. Usually they live there, feeding on the dead matter of the surfaces. At times they work down into the pores of the skin and manifest themselves as blackheads, or as the more painful affliction—boils. Normally the infant enters the world free from bacteria, but bacteria soon settle on his skin. They penetrate the nose and mouth; the first respiratory movements and cries carry them into the respiratory passages; and between the tenth and seventeenth hour they have reached the intestines. From the minute of birth until the time of death there is a constant struggle between the individual and the microbes, and one individual has jokingly remarked, "Microbes get us at last." This is strictly true, for most individuals die of bacterial diseases long before the allotted three score and ten years have elapsed, and the bodies of even those who die from accident or old age, unless cremated, are decomposed by bacteria.

Ordinarily, the deeper respiratory passages contain few bacteria, but it has been proved that at times even the tuberculosis bacilli can penetrate with the inspired air to the bottom of the lungs.

On account of its acidity, yeasts and molds flourish better in the stomach than do bacteria. However, at least thirty different kinds of bacteria have been described as occurring in the stomach. Many of these have attracted special attention on account of the belief that their presence may favor other more injurious species.

The intestines, on account of their alkaline reaction and the partly digested condition of their contents, are a great reservoir of bacterial activity. In the upper part there are few, but by the time the descending colon is reached billions of bacteria are present. Sometimes they constitute one third of the total dry contents of the intestine. The health of the individual is deter-

mined by the number and kind of bacteria, and at least one writer has suggested that our very personality is governed by the microbes we carry about with us.

The normal tissues and the blood of animals are usually free from bacteria. Micro-organisms are rarely found on certain healthy mucous membranes, such as those of the kidneys, bladder, and lungs. Occasionally they pass through the skin or mucous membranes of the digestive tract after which they may be found for a short time in the blood. This is especially true during the height of digestion, and we find at this time large numbers of white corpuscles swarming into the intestinal mucosa ready to pick up and devour any bacteria which may enter. In this manner the body is protected against invaders. In certain diseased conditions the blood and tissues of man and lower animals become filled with bacteria. Just before and soon after death they rapidly invade and tear down the body.

Functions of Bacteria.—We are living in a world teeming with bacteria. They cover the surface of the body and swarm in the alimentary canal. Hence, we are prone to ask: Do those in the alimentary canal help us, or would we be better off without them? Early experimenters found that chickens, frogs, and turtles if hatched free from bacteria and fed on sterile food did not grow normally, whereas, other experimenters have found that the guinea pig could be reared to maturity on sterile food with its alimentary canal free from bacteria. Still more recent investigators claim to have found the digestive tract of the white bear, seals, reindeer, eider ducks, and penguins in the Arctic regions to be entirely sterile; hence, bacteria are not essential to these animals. They may assist cows, sheep, and horses in their digestive processes, but it is doubtful if they are essential in the alimentary canal of man. Their real significance comes in the fact that we are living in a world filled with them. They cannot be kept out of the alimentary tract. The normal flora does no harm but does produce products which are inimical to the growth of many injurious ones. As a consequense of the knowledge of this fact, considerable attention has been given to the favoring of the beneficial bacteria in man. The good effect which sometimes follows the use of sour milk and buttermilk is due to their carrying into the intestines beneficial bacteria together with food favorable to their growth. Sometimes individuals are given cultures of *Lactobacillus bulgaricus*, or still better, *Lactobacillus acidophilus*. Little is to be gained by this procedure unless at

the same time the diet of the individual is made to favor these species.

The great Russian bacteriologist, Metchnikoff, claimed that the rate with which man ages is determined not by the years he has lived, but by the bacteria which inhabit his digestive system; and Morris, in his interesting book "Microbes ond Men," even argues that they control the personality of man. They are the governors of his whims, fancies, likes, and dislikes. Just as alcohol partaken of by man usually causes first elation, later depression, and finally helplessness, likewise bacteria at first invigorate and later depress. All are familiar with the statement: "I never felt so well in my life as I did just before I came down with this sickness; in fact, I felt unusually well and spoke to my friends about it." Parents are continually telling the doctor: "My boy was never so full of life as the day before he came down." Dr. Morris further argues that the optimist, the pessimist and the neurotic are what they are, melancholic and morose because of the bacteria which they carry about. A rabbit given a certain small dose of strychnine will show no ill effects if left quietly by itself, but if such a rabbit be disturbed it goes into convulsions. Likewise, the neurotic made so by the absorption of bacterial poisons, if left quietly by himself is docile and contented, but if slightly irritated becomes morose, sullen, cross or even pugnacious, depending upon circumstances.

Bacteria play numerous wonderful parts in the many changes going on in this world. This is true to such an extent that it is hard to conceive of our living in a world without bacteria. Examining the soil from which man draws—either directly or indirectly—his clothing, food, and other necessities of life, we find it to be a veritable garden of minute plants which have been at work within it long before man began to till the soil. What a wonderful change they produce in transforming the bleak, barren rock into a fertile field from which man can gather his delicious berries, or beautiful flowers; his golden sheaves of wheat, or his deep green nutritious loads of hay!

Changes in temperature cause small cracks to appear even in the more resistant rocks. Bacteria being of microscopical size soon penetrate these small crevices, and produce carbonic, nitric, and other acids which gradually dissolve the rock. The microorganisms attack even the most minute fragments reducing them continually to smaller and smaller particles. If one picks from a fertile soil a speck of rock, it will be found coated

with a fine film of these minute plants. They have drawn on it for their food, and at the same time they have rendered it soluble so that the wheat, alfalfa, or corn plant can feed upon it. Bacteria continue their work long after the rocks have been changed to soil. Each day they liberate a little more plant food for the growth of the crop. During the year they are able in a fertile soil to liberate enough plant food for the production of a good crop. When manure is applied to a soil it not only supplies food for the growing plant, but it also carries to the soil beneficial bacteria and supplies those already there with food. These in turn liberate a little more iron, sulphur, potassium, or phosphorus, and the farmer reaps from his soil a bigger and better crop.

One of the essential elements for plant production, and the one which is usually in the soil in the smallest quantities, is nitrogen. This, unless it be applied to the soil in the form of the expensive fertilizer known as nitrates, must be prepared for the plant by bacteria. The micro-organisms that carry on this particular form of work are the true kitchen maids of the soil, and the farmer finds his crops measured directly by the speed with which they prepare the meal. If they are active and prepare the right food, other things being favorable, there will be a good crop; but if they fail in their work, though everything else may be ideal, there can be no crop.

An examination of soils has shown that those which are most fertile contain the greatest number of beneficial bacteria. If the soil is rich in plant residues and has the right quantity of water and sufficient heat many bacteria will be found there changing the plant residues into gases and acids which act upon insoluble substances and render them soluble. One group of bacteria changes the plant and animal proteins into ammonia, and their activity can often be detected by the odor of ammonia coming from stables or manure piles.

However, most plants cannot use nitrogen in the form of ammonia; it must be changed into nitrates. This is done by two species of bacteria. One group feeds upon ammonia and manufactures nitrous acid. Nitrites are poison to plants; hence, if these were left unchanged at this stage we would have no plants; but it so happens that another class of bacteria feeds upon the nitrites as fast as they are formed and yield for us nitric acid. This reacts with the various minerals of the soil. This form of nitrogen is now ready to be taken up by the growing plant and manufactured into nourishing food, beautiful

flowers, delicious fruit, or fragrant perfumes for the human family.

So far only the plant food already in the soil and the changes through which it passes have been considered. However, the farmer is concerned with the substances his soil lacks and which must be added in order to get good crops. In many cases the lacking element is nitrogen. One notes from fertilizer quotations that this element costs 15 cents a pound or over if purchased in the form of sodium nitrate, ammonium sulphate, or dried blood. On making a simple calculation we find that at this price it would cost $15.00 for enough of these substances to produce 100 bushels of corn; $11.00 for enough to produce 50 bushels of wheat; and $7.50 for enough to produce 1 ton of alfalfa hay.

In these calculations it has been assumed that one recovers in the form of corn, wheat, or alfalfa every pound of the commercial nitrogen which has been applied, which as a matter of fact is an utter impossibility; hence, we have to look elsewhere to obtain nitrogen for our growing crop. Here also bacteria come to the rescue.

There are 75,000,000 pounds of atmospheric nitrogen resting upon every acre of land. However, none of the higher plants has the power of taking this directly out of the air. One family of plants, the Leguminosae, which includes peas, beans, alfalfa, clover, and many others, if properly infested by bacteria, has the power of using this atmospheric nitrogen. Under this condition and with these plants nitrogen no longer remains the limiting element of crop production, for the bacteria which live within small nodules upon the alfalfa are master chemists. Within their tiny laboratory they can bring about changes which man can imitate only imperfectly with costly machinery and powerful electric currents. In some of the experiments carried on at the Illinois Experiment Station these minute organisms were found to increase the value of the annual crop of alfalfa hay $37.50 an acre. That is, considering the nitrogen in the alfalfa at the price we would have to pay on the market for an equivalent quantity of nitrogen in the form of a commercial fertilizer! If these crops were plowed under, the fertility of the soil would be increased to just that extent. One writer has said of them: "They not only work for nothing and board themselves, but they pay for the privilege." This is strictly true, for all they require is a plant on which to grow and a well aerated moist

soil containing limestone as well as the essential plant food other than nitrogen. They cannot work in an acid soil nor can they produce something from nothing.

There is another class of nitrogen-gathering organisms within the soil which differs from the above in that it lives free in the soil and gathers nitrogen. Under ideal conditions these organisms may gather quite appreciable quantities.

It is quite possible that much of the benefit derived from the summer fallowing of land is due to the growth within the soil of this class of organisms which store up nitrogen for future generations of plants. It has been discovered that they are more active and found in greater numbers in soil treated in this manner. All of the work which the farmer puts upon the soil in rendering it more porous reacts beneficially upon these bacteria, because they not only love atmospheric nitrogen and oxygen, but because they must have them.

Bacteria are the universal scavengers that lick up the dead bodies of plants and animals as completely and nearly as rapidly as fire. Were it not for these organisms the world in time would be filled with never-changing organic matter. The plant residues, trees, and animal bodies would remain in the soil, and with them the carbon which is required by the green plant. Bacteria, in getting their required energy, are continually liberating carbon, thereby permitting this important element to start again on its constructive journey. If carbon and nitrogen could but speak, what wonderful tales they would tell! The chemist, the bacteriologist, and the farmer each would be wiser, for many of the changes through which carbon and nitrogen pass due either to the action of the one-celled plants, bacteria, or the higher plants, wheat and alfalfa, are so complex that the scientist even with his apparently magical methods cannot follow them.

There is a theory that bacteria are the architects which have built and are building many of the chalk deposits of the world. Certain bacteria which live in the warm portions of the sea effect the reduction of nitrates in the water to ammonia. The ammonia combines with carbon dioxide, produced by other bacteria. This reacts with calcium sulphate in the sea water with the formation of calcium carbonate or chalk. This product separates out in the form of small spherical or elongated grains, and accumulates in deposits known as oolite. Such deposits are being formed today in great abundance off the coast of Florida. Prob-

ably in early geological ages this method of chalk formation was very important.

The plumber at times meets hard masses of rust-like deposits which partly or completely fill the water mains. To him these are a curiosity or a nuisance, but to the bacteriologist they recall the method by which some of the iron deposits were formed. In the past some of the warm waters covering the surface of the earth carried large quantities of iron salts. Iron bacteria grew in these waters and extracted from the water the iron. This they deposited in a coating that surrounded their bodies, a sheath. When they died the iron was deposited; thus, we have the origin of at least part of the bog iron ore of today.

Bacteria are found by the thousands in every drop of water in the cesspool where they lie in wait and quickly devour the material which enters. Paper, bone, and wood are quickly changed into gases and ash. The repulsive poisonous substances soon disappear under their magic wand, and it is only a short time until the water again becomes pure.

Bacteria grow in cream, making churning easier. They give to butter its characteristic flavor. They cause the ripening of cheese; thus, giving to it the peculiar flavor in opposition to the insipid taste of the fresh cheese.

Some writers even argue that the disease-producing microbes are beneficial, for these slay plants and animals; consequently the materials of which their bodies are composed may be used by others. It is, however, more logical to consider that they formerly were harmless, or that they were beneficial, as they grew on the surface of plants and animals, and decomposed dead material. It can well be imagined that they later reached and were carried about on the bodies of animals and still later started to invade the body. In the struggle between them and their host some were routed and disappeared, while others became true parasites preying upon plants and animals. These are now the outlaws in bacterial society. Such bacteria produce poisons which are injurious to man or lower animals, but man is learning to turn their poisons against themselves in the production of vaccines and serums which are used to render man and the lower animals immune to their attacks.

These are only a few of the many ways in which bacteria benefit the human race. They are at work in the silo, rendering the food more palatable and nutritious for cattle. They give to sauerkraut and pickles their flavor. They take part in the

tanning of skins, the retting of flax, the curing of tobacco, the flavoring of coffee and cocoa, and the making of indigo from the indigo plant. In short, they help us in a thousand and one ways which we little suspect. Even today only a few of the changes have been fully studied; hence, one of the most instructive and interesting tasks set for man is to learn what these processes are and how to govern them.

CHAPTER VI

MORPHOLOGY OF MICRO-ORGANISMS

EVERYONE recognizes that there are different races of mankind. Even the untrained naked eye perceives different varieties of plants and animals. The lynx-eyed lens has revealed to the scientist bacteria differing in form and structure. The branch of bacteriology which deals with the form and structure of micro-organisms is known as morphology.

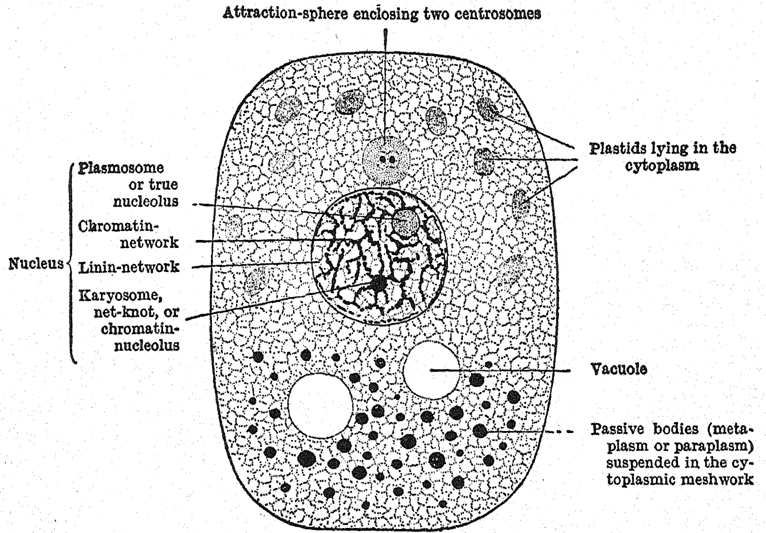


Fig. 18.—Diagram of a typical cell. (*Wilson.*)

The Cell.—One can divide an animal into different parts as head, trunk, arms, and legs. These in turn can be further divided into eyes, ears, nose, and so on. Now, if one examines with a microscope any of these parts he finds that they in turn are composed of still smaller parts called cells. Therefore, plants or animals, man or the microbe are all composed of a cell or cells. These cells vary greatly in shape. Some are cylindrical;

others are oval; still others are globular. In size they vary from the tiny bacterium which requires a great magnification before it can be seen, to the muscle or nerve cell which is measured in inches or even feet. If one were to examine more minutely a cell it would be found to be surrounded by an outer retaining membrane—the cell wall. Just beneath the cell wall in the majority of cells is a supporting structure of less dense material—the ectoplasm. Still deeper within the cell is a clearer less refractive part containing besides granules of food and waste, many cell organs. This is known as the endoplasm. Within the center of the cell is a still more dense portion which stains with the aniline dyes more deeply than the rest of the cell. This is the nucleus. The cell, then, is the unit of life and

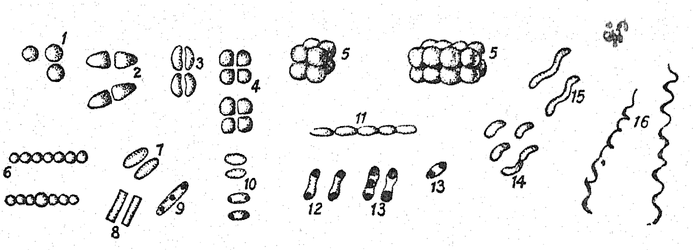


Fig. 19.—The normal types of bacteria: 1–6, cocci; 7–13, bacilli; 14–16, spirilla; 1, micrococcus; 2 and 3, diplococci; 4, tetracoccus; 5, sarcina; 6, streptococcus (the lower chain includes an arthrospore); 7 and 8, bacilli; 9, 10, 12, 13, bacilli with various granules; 11, streptobacillus; 14, vibrio; 15, spirillum; 16, spirochaeta, treponema. (*From Kendall; Bacteriology. Published by Lea and Febiger.*)

may be defined as a mass of protoplasm containing a nucleus. However, when one includes the bacterial cell he finds it necessary to modify this definition so as to read "a cell is a limited mass of protoplasm containing nuclear material." The reason for this modification is that bacterial cells probably have their nuclear material diffused throughout the body of the cell; and as a consequence, the definition for bacteria must make clear that they are unicellular micro-organisms devoid of a definite organized nucleus.

Shape of Bacteria.—Bacteria are minute single cell organisms which may occur free or in larger or smaller aggregates. Although there are thousands of different bacteria having great variations in properties, yet they can be roughly grouped under three basic forms; cylindrical, globular, or spiral. The cylin-

drical or rod-shaped bacteria may be likened unto an unsharpened lead pencil. Some have rounded ends, others straight,

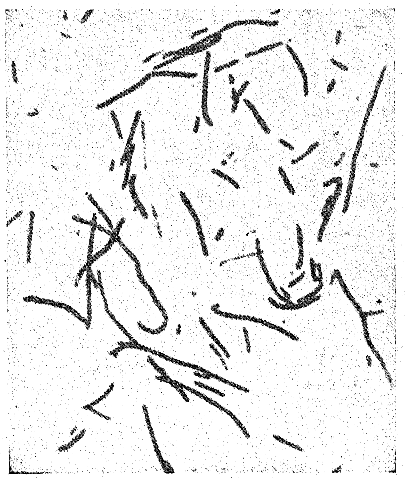


Fig. 20.—Large bacilli. (*After Harrison.*)

while still others have the ends hollowed out. The size also varies, some being so small that it is impossible to determine

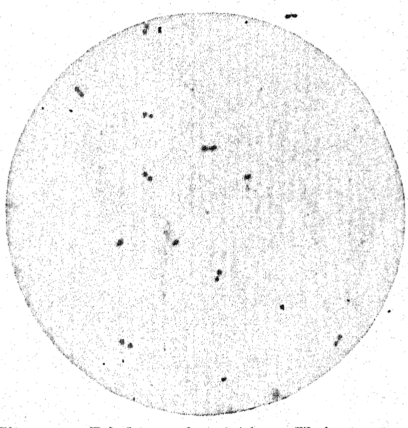


Fig. 21.—Diplococci. (*After Heinemann.*)

even with the best microscope whether they are cylindrical or globular organisms. All rod-shaped bacteria are known as bac-

illi (*singular,* bacillus). The term "bacterium" signifies a rod or little stick and is now used to refer to the entire group of bacteria as well as to members of a particular genus.

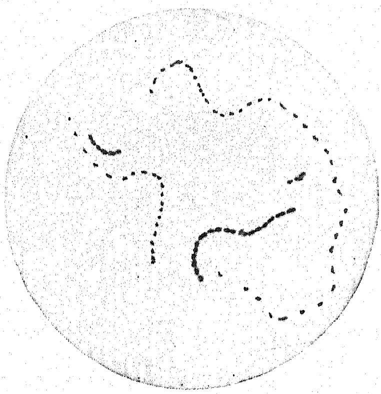


Fig. 22.—Streptococci. (*After Heinemann.*)

A second type of bacteria is the globular which may be likened to minute berries or at times to an egg. They are known as cocci (*singular,* coccus). Often when two remain connected we

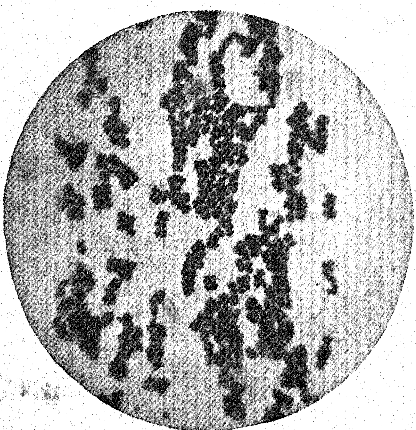


Fig. 23.—Sarcina. (*After Gruber.*)

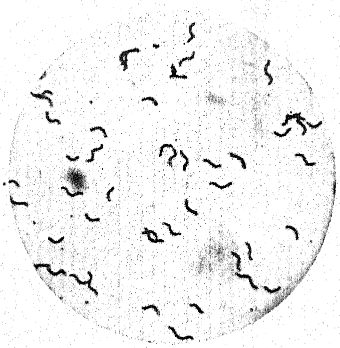


Fig. 24.—Spirilla. (*After Omiliansky.*)

obtain a coffee-bean-shaped organism, or at times an organism similar to the head of a lance. The cocci may be large or small and group themselves in various ways.

The third group of bacteria are the spirilla (*singular*, spirillum), which may be likened to a corkscrew. The spiral may be loosely or tightly coiled. There may be one, two, or more coils. At times the organisms may be so small and the curve so slight that the bacterium viewed under the microscope appears to be "comma-shaped."

Roughly speaking, there are about three times as many bacilli known as cocci and five times as many cocci as spirilla.

Cell Structure.—Many bacterial cells are surrounded by a gummy mass called the capsule. In stained cultures this appears as a halo surrounding the bacteria. The presence of a great number of these capsulated bacteria in milk gives to it a ropy consistency. When this condition is present the outer

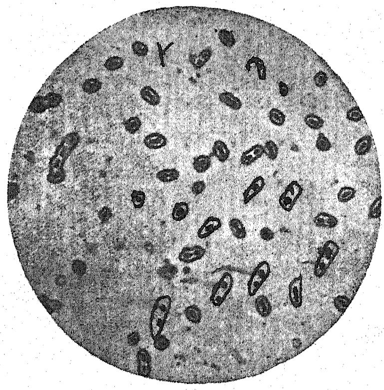


Fig. 25.—Capsulated bacteria. (*After Buerger.*)

coats of the bacteria stick together permitting the milk to be drawn out in long threads. When these capsulated forms get into flour or yeast they usually give to the bread a putty-like appearance.

There is a greater tendency for bacteria to form capsules when grown in body fluids than when grown on other media. The anthrax bacillus inoculated into the rat or lizard surrounds itself with a capsule, and hence, becomes very resistant. The capsule appears to be a defensive secretion and usually capsulated organisms are more likely to infect than similar non-capsulated organisms.

Frequently the bacterial cells are found collected in masses, the bacterial bodies appearing to be embedded in the gelatinous

substance. To these aggregates the name zooglea has been given. The mother of vinegar is such a zoogleic mass. Zooglea frequently occur in soil, sewage and decomposing food.

A sheath is produced where bacteria grow in chains secreting a firm membrane about the whole filament thus forming a tube in which the organisms occur. Various substances such as iron or calcium may be deposited in this sheath. The iron bacteria are prone to form sheaths.

Bacteria have a cell membrane through which the food must pass and which retains the liquid of the cell within bounds. Lining the cell wall on the inner side is a dense layer of protoplasm which has a selective action on the food taken up by the

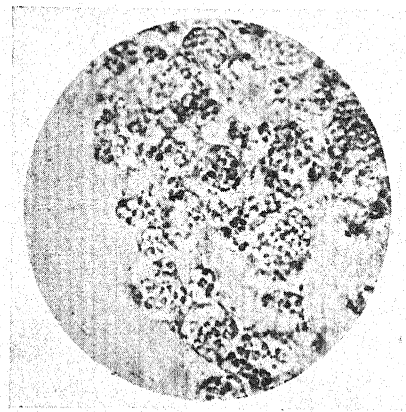


Fig. 26.—Zooglea formations of the nitrobacter. (*After Winogradsky.*)

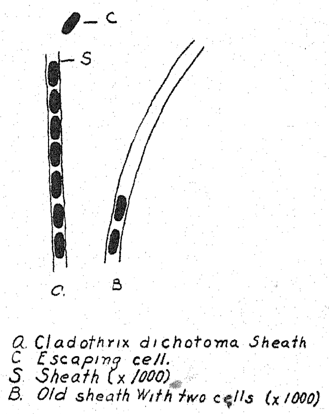


Fig. 27.—Illustrating bacterial sheath. (*After Ellis.*)

cell. If the cell is placed in a strong sugar or salt solution, the cell shrinks and the ectoplasm is torn from the cell. This is known as plasmolysis. If the bacteria are immersed in distilled water they swell and may burst, the cytoplasm passing out into the surrounding medium. This is referred to as plasmoptysis.

In the cells of higher plants and animals is a distinct structure enclosed in its own membrane which stains deeper than the surrounding protoplasm and is known as the nucleus. Bacteria stain more uniformly throughout the whole cell, consequently it is debatable whether bacteria in the true sense possess a nucleus, but some authorities teach that the greater part of the cell inclusion is nuclear material. Scattered throughout the cell

may be seen dark granules. These bodies, termed metachromatic granules, may react to stains in such a way as to differentiate them from the rest of the cell and give us information as to their composition. They are probably the reserve food of the cell.

Movement of Bacteria.—If one places a handful of hay in a bottle containing beef tea and allows it to stand in a warm place for twenty-four hours and then examines a drop of the infusion in a hollow slide under the microscope, it will be found to be filled with living micro-organisms. One notices that all of the particles within the drop are moving—even the smallest particles of hay. There is a great difference, however, in the

Fig. 28.—Bacteria with flagella on one pole. (*After Gruber.*)

way in which they move—some swing back and forth, while others are seen to move swiftly across the field and out of sight All of the bodies which are thus seen to move rapidly are micro-organisms which have the power of movement. This action is known as vital movement. By appropriate methods it has been shown that the motile bacteria have on their bodies long hair-like appendages called flagella. These may be situated at one or both ends, or they may even surround the entire body of the organism.

An organism with a flagellum at one end of the cell is *monotrichous;* one with two or more flagella at one end is *lophotrichous;* an organism with a cluster at each pole *amphitrichous,* and one with flagella surrounding its body, *peritrichous.* It is

by the striking of the medium with the flagella that the organisms are able to move. Young cultures are in general more

Fig. 29.—Bacteria with flagella on both poles. (*After Harrison.*)

actively motile than are old cultures, hence are usually selected for the demonstration of flagella. In spore-bearing organisms the young vegetative rods which have just emerged from the spores

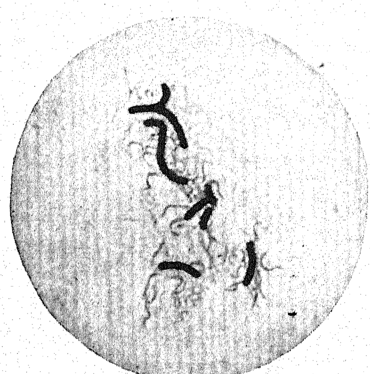


Fig. 30.—Bacteria with flagella surrounding the body. (*After McBeth.*)

are actively motile, the flagella having been developed during the first few hours of vegetative life. This period in the life history of the organism is referred to as the swarming stage.

Bacterial movement shows great variation. "It may be straight, or wiggling, or gyrating. Short rods often tumble about, appearing temporarily, when in an upright position, like small cocci. Spiral forms are whirling like ships' propellers. The aspect becomes especially lively when in a drop of putrid liquid (old liquid manure, for instance) the motile bacteria are chased around by bacteria-hunting protozoa."

If one adds water to a bit of clay or a drop of Chinese ink and examines it in a hanging drop under the microscope, the particles will be seen in rapid motion. This fact was carefully observed and vividly portrayed for gold particles by the inventor of the ultramicroscope. "The tiny gold particles did not swing stationary in the water; they moved with astounding rapidity. He who has seen a swarm of flies dancing in the sunlight can obtain a notion of the motion of these tiny gold particles; they hop, dance, spring, crash together and fly apart so rapidly that the eye can hardly make out their movements." This form of movement is manifested by all very small particles when in suspension. It is not due to life but results from the bombardment of the small solid particles by the molecules of the liquid. This was first described by the English botanist, Robert Brown, nearly a century ago; hence, it is known as brownian movement. Bacteria in common with all other small particles manifest brownian movement. The bacteriologist learns to distinguish between it and the vital movement described above.

As one examines moving bacteria under the microscope one would think that they were moving with the speed of an express train, but on actually measuring their speed it is found to be an illusion. The cholera organism has been known to attain for a short distance the enormous speed of 8 inches an hour! That is, they travel in relation to size at about the same speed as does a running man.

Reproduction.—Bacteria quickly decompose the body of an ox. Plants and fruits quickly decay because of them. Millions of tons of organic matter are carried to rivers, lakes, and oceans each year. Bacteria change all of this into residual ash and gaseous products. They modify the whole surface of the earth. One may wonder how it is possible for such small plants to accomplish such gigantic tasks. This is due to the rapidity of multiplication. The bacilli grow until they have reached a certain size, when they divide into two daughter cells. These

in turn grow to maturity and likewise divide. At times the various cells on division may remain connected, and have the appearance of a chain. Such a chain of organisms is called streptobacillus.

The cocci may divide in one, two, or three planes. When they divide in the same plane and the daughter cells touch at one side only and have the appearance of closely strung beads, they are known as streptococci. They may divide in one plane followed by division in another at right angles to the first, thus forming a sheet of cells. These are the micrococci. Others may divide in one plane followed by a second division which is not always at right angles so that the organisms form an aggregate

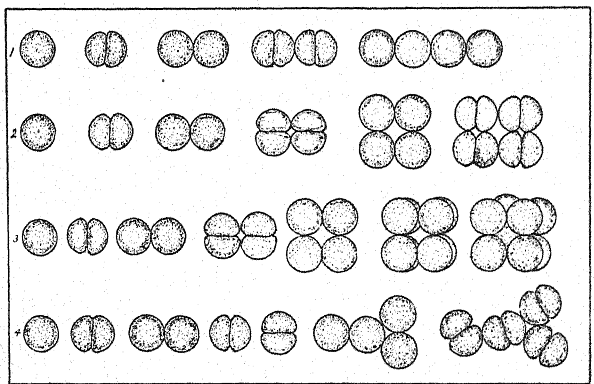


Fig. 31.—Forms of division and grouping in the cocci. 1, Streptococcus; 2, micrococcus with cells arranged in tetrads; 3, sarcina; 4, staphylococcus.

which has the appearance of a bunch of grapes. These are the staphylococci. Still other organisms may divide in three planes, thus giving rise to packets of cells—a condition similar to that which one would have if four marbles were placed on a level surface and four others placed upon them. These are the sarcina and have the appearance of a bale of cotton.

A bacterial generation is taken as the time required for a mature cell to divide and the resulting daughter cells to reach maturity. This process may be completed in one-half hour; at times, even more rapidly. Under less favorable circumstances it may be much longer. It has been estimated that if bacterial multiplication went on unchecked the descendants of one cell

would in two days number 281,500,000,000 and in three days the descendants of this single cell would weigh 148,356,000 pounds! It has been further estimated by an eminent biologist that if proper conditions could be maintained for their life activity, in less than five days they would make a mass which would completely fill as much space as is occupied by all of the oceans on the earth's surface, considering the average depth of the water to be one mile.

Even in the face of these assumptions one need not fear, for bacteria have been on this earth and have been multiplying probably long before the advent of man, and as yet the earth has not been filled by them. This is due to there being a struggle among them just as there is among higher plants and animals. One knows that if wheat is sown too thickly, none of it matures. Sometimes it is a lack of food, other times a lack of sunshine, and at still other times it is a lack of moisture which prevents its growth. So it is with bacteria. The food or water may give out; but, more often it is the bacterial products which have accumulated and prevent their multiplication.

Longevity of Bacteria.—A bacterial generation extends from one cell division to the next. Many bacteria in young cultures are larger and more sensitive to heat and chemicals than are old cultures, consequently some bacteriologists have argued that the newly formed cells pass through a period of physiologic youth, maturity and old age. However, we could not look upon death as coming to a multiplying culture from old age for due to their method of multiplication both daughter cells are similar in age and composition. It is well known that many bacteria while in the spore condition can survive for a quarter of a century. They have been obtained from soil which has been kept under air-dry conditions in fruit jars for over fifty years. Probably they may live for centuries.

Size.—The unit of measurement in microscopical work is the micron. It is one thousandth of a millimeter, or approximately one twenty-five-thousandth of an inch. It is represented by the symbol μ. The unit of measurement in ultramicroscopical work is the millimicron $m\mu$, which is one thousandth of a micron. Most bacteria are from 0.5 to 5 μ in length. Some are so small that they cannot be seen with the microscope. Others may be even 30 or 40 μ in length. They are smallest in the case of the cocci and largest in the case of the spirilla.

Although there is a great variation in the size of bacteria, all

are extremely small. Even the largest are not visible to the naked eye. The smallest are beyond the range of our most powerful microscopes. The Pfeiffer bacillus, the organism which was thought to cause influenza, is one of the smallest well-known organisms. It is a rod-shaped organism, and if placed end to end 125,000 of them would be required to make 1 linear inch. It would require 15,000 typhoid bacilli to reach an inch. The organism causing relapsing fever is one of the largest known, and of these some 1500 would be necessary to reach 1 inch. We often magnify bacteria one thousand times, and then they appear as dots under the microscope. If we were to magnify man to this extent, he would be a giant indeed—6000 feet tall and 1500 feet wide! In a sample of milk containing 1,000,000,000 bacteria in 1 cc. there is less than one thousandth of its volume bacteria, while a little globe of bacteria no larger than a drop of water would consist of 50,000,000,000 bacteria.

It is probable that there are many ultramicroscopical organisms much smaller than those which can be seen with the aid of the microscope. They are known from the changes which they cause. Their size can be estimated by filtration, consequently they are referred to as filtrable viruses. They are parasitic; producing disease in man, the lower animals, and plants, possibly including bacteria.

Weight of Bacteria.—Knowing the size and density of bacteria, it is a comparatively simple matter to calculate their weight. Assuming the average bacterium to be a cylinder 2 μ long and 0.5 μ in diameter, the volume of such an organism would be 0.000,000,000,39 c.mm. If it had a specific gravity of 1.2 its weight would be 0.000,000,000,471 mg., or in other words, it would require approximately 2000 million of these to weigh a milligram, or thirty thousand times this number to weigh 1 ounce. It is the great number which makes them so effective. For example, 1 acre-foot of soil weighs 3,600,000 pounds. In each gram of soil there may be as many as 50,000,000 bacteria. This would mean that in 1 pound of soil there are 23,000,000,000 bacteria! Then

$$\frac{50,000,000 \times 454 \times 3,600,000}{2,122,000,000 \times 1000 \times 454} = 90 \text{ pounds}$$

the weight of bacteria in 1 acre-foot of soil.

Spore Formation.—When adverse conditions arise, especially a lack of water, many bacteria have the power of mobilizing the

vital parts of their bodies into much smaller space than is occupied during their normal life. In order to do this they exclude all excess moisture and surround themselves with a tough resistant coat. In some respects this form of the organism resembles the seed of the higher plants, and we speak of it as the spore. While in this stage the organisms can withstand many conditions which would quickly prove fatal to the growing or vegetative forms of bacteria. Some of them while in this condition can withstand the temperature of boiling water for many hours, or they may survive treatment with strong carbolic acid. For the time being they lose the power of multiplying, but they are still alive, and if brought into appropriate surroundings they will change into normal bacteria just as the kernel of wheat changes into a young plant when placed into moist soil.

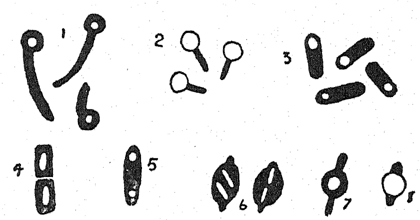


Fig. 32.—Types of sporangia and spores in bacteria. 1, Spirillum with a terminal enlarged spore; 2, bacillus with a terminal enlarged spore; 3, bacillus with a terminal spore, not enlarged; 4, bacillus with an equatorial spore, not enlarged; 5, bacillus with two small terminal spores; 6, bacillus with two elongated spores; 7, 8, bacilli with equatorial swollen spores. (*After Buchanan.*)

It is indeed fortunate for mankind that but few of the disease-producing organisms form spores. However, there are many of the bacteria such as those that cause fruit, meat, and various other food products to spoil, which do form very resistant spores. That is why many food products have to be heated for such a long time or to such high temperatures to make them keep. At times, in order to avoid heating the substances to a high temperature, the intermittent method of sterilization is used. In this method the substance which is to be sterilized is heated to boiling for a short time. This heating kills all of the organisms which are not in the spore form. After standing in a moderately warm place for twenty-four hours, during which time the spores vegetate, it is again heated for a short time, thus killing the others which have started to grow. Often this is repeated

even a third time before the vegetables or other products are stored.

The manner of formation of the spore within the body of the organism is intensely interesting, for it varies with different species. The bacteriologist has devised means whereby he can color the body of the organism one color and the spore within the body a different color. In some instances if we stain the various organisms in the first stages of spore formation, we find

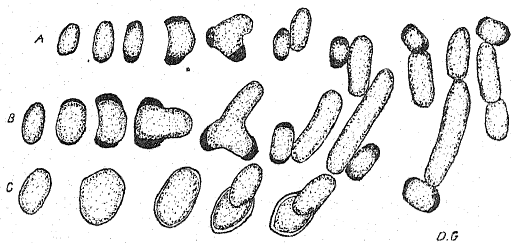


Fig. 33.—Illustrating spore germination.

the little red dot situated within the center of the blue body. In others, it is also in the center, much wider than the body of the organism; hence, we have a boat-shaped organism or clostridium. In still other bacteria the spore forms at the end, and we have the drumstick-appearing organism, or capitate. This is the case with the organism causing lockjaw. Spores when brought into appropriate conditions germinate. The process of germination varies in different bacteria.

CHAPTER VII

YEASTS, MOLDS, AND ACTINOMYCES

Closely related to bacteria and often intimately associated with them are yeasts, molds, and actinomyces. Being devoid of chlorophyll, they, like bacteria, are dependent upon the higher plants and animals for their energy. This they obtain by the splitting of large molecules into smaller ones, many of which are of economic importance. Some yeasts, and especially molds and actinomyces, are widely distributed and often try the patience of the bacteriologist, as obnoxious weeds, contaminating his cultural media. Bacteria, molds, and actinomyces are the trinity which liberate plant nutrients and which, together with yeasts, cause food spoilage. Yeasts and molds are even more important than bacteria in the production of organic compounds of commercial importance. They play a major rôle in the ripening of some food products and are not without interest from a public health standpoint.

YEASTS

Yeasts are unicellular colorless plants having a definite organized nucleus and reproducing asexually, usually by budding; they may form ascospores. Most yeasts are larger than bacteria, the more common being from 3 to 10 μ by 3 to 100 μ and varying greatly in shape, a factor which is made use of in their classification. The round or globular isolated cells are referred to as *cerevisiae*, the elliptical cells as *ellipsoideus*, the sausage-shaped cells as *pastorianus*, and the lemon-shaped cells as *apiculatus*. Ordinarily, the cells are isolated or united in small groups, especially in old cultures, occasionally the cells remain united in long chains having the appearance of a rudimentary mycelia. These moldlike structures in the early study of the subject caused confusion in their classification. The *Mycoderma* species are typical film producers, a structural peculiarity which tends to keep them at the surface of the liquid medium, thus enabling them to obtain the necessary oxygen.

Cell Structure.—Yeasts have all of the parts usually occurring in cells and consequently are typical cells. They are surrounded by a thick easily demonstrated cell wall composed of

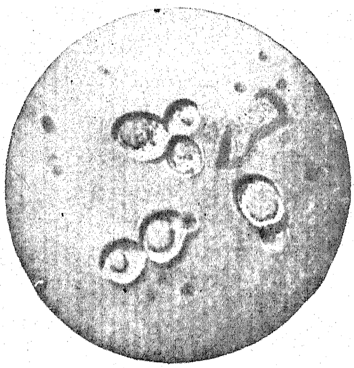


Fig. 34.—Yeast cells showing budding. (*After Van Hest.*)

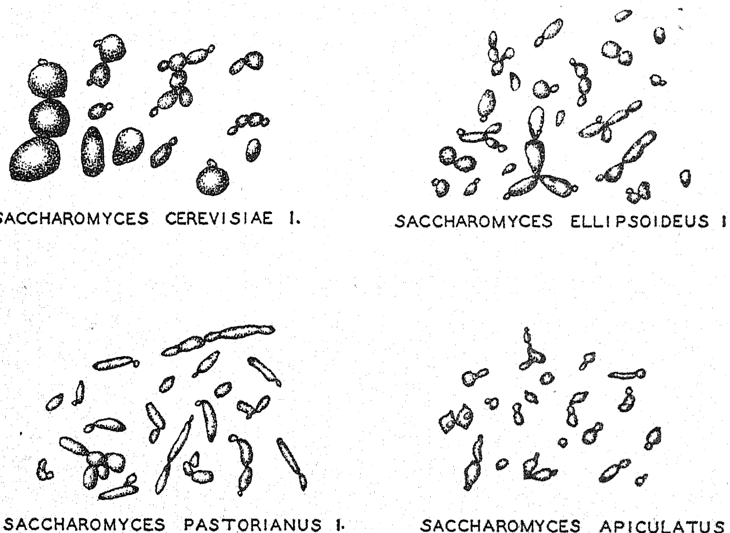


Fig. 35.—Various types of yeast cells. (*Redrawn from Hansen.*)

yeast cellulose. The membrane thickens in older cells and may be surrounded by a gelatinous capsule similar to that possessed by some bacteria. They do not possess flagella and hence are nonmotile. They possess definitely organized cell nuclei which

are readily demonstrated by proper staining methods. In the young cell the nucleus is usually globular but may vary in shape as the cells mature. In reproduction the nucleus first divides, part going into the bud and the balance remaining with the mother cell. Within the yeast cells are less refractive bodies, vacuoles which are filled with a clear liquid. Vacuoles increase in number, due to a lack of food or exhaustion of the cell. Scattered throughout the cytoplasm even in the vacuoles are refractive granules. These probably represent reserve food supply and consist of glycogen, oils, and even protein-like substances. Often the vacuoles contain a small granule which exhibits an active brownian movement, referred to as the "dancing body."

Methods of Multiplication.—Yeasts reproduce vegetatively and by means of spores. Vegetatively they reproduce by budding and by fission. The characteristic method is by budding.

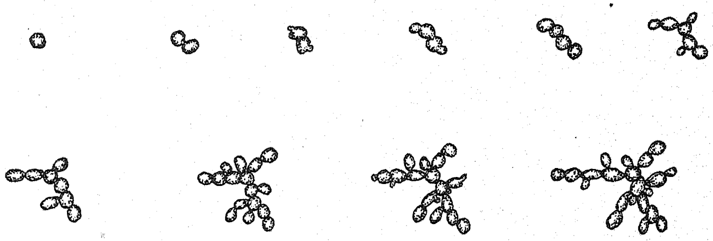


Fig. 36.—Illustrating yeast multiplication by budding.

The bud first appears as a small protuberance separated from the mother cell by a very narrow collar and increases in magnitude until it has acquired a certain size upon which it separates from the mother cell. The daughter cell grows rapidly and soon equals the mother cell in size, after which it in turn forms buds. When multiplication is very active, each cell may form many buds on different parts of its surface at the same time. It not infrequently happens that buds, while still attached to the mother cells, commence to form buds on themselves. This gives rise to a small colony of cells.

Some species of yeasts multiply by fission much as do bacteria. The cell matures, after which a cell wall forms and divides the original cell into two daughter cells. The rapidity with which yeasts multiply depends upon the species, the medium, temperature, and aeration. The best medium contains beer wort. Yeasts multiply between 0° and 50° C.; the op-

timum varies with different species, but it is usually between 25° and 30° C. Aeration of the culture liquid is one of the foremost factors in accelerating vegetative increase.

Spore Formation.—All true yeasts produce spores. In the majority of yeasts the spores are formed by a repeated division of the nuclei, around which the protoplasm condenses with the formation of spore membranes. The spores are formed within a spore sac, and a cell containing spores is called an ascospore. Some yeasts develop only one spore; others produce from two to eight; consequently, yeasts may multiply by spore formation. The spore shape varies, the most frequent being spherical with a tendency to produce ellipsoid-shaped spores. Certain species produce kidney-shaped spores, whereas others produce hat-shaped spores or spores shaped like the segment of a sphere.

Bacteria produce spores under adverse conditions, but with yeasts it is different. Hansen has formulated the following rules for the production of spores by yeast: The cells should be well nourished and healthy; they should be well aerated; there must be an abundance of moisture and a temperature of about 25° C. Most yeast spores are destroyed by the temperature of pasteurization and hence are less resistant than bacterial spores.

Classification.—Yeasts may be divided into two great groups—true yeasts and false yeasts. True yeasts form spores, whereas the false yeasts do not. A convenient classification of the more important yeasts is as follows:

I. *Saccharomyces Cerevisiae.*—These are the yeasts generally used in the manufacture of bread and alcoholic liquors and may be divided into three groups.

(*a*) Bottom yeasts of German beers.

(*b*) Top yeasts of English beers.

(*c*) Distiller's yeasts, which produce larger quantities of alcohol than the beer yeasts.

II. *Saccharomyces Ellipsoideus.*—These yeasts occur naturally on the grapes in the vineyard and are sometimes called wild yeasts. They are used to some extent in the making of wines and distillery products. In some countries they are used in breadmaking.

III. *Tolura, Pseudo or False Yeasts.*—These are the trouble-makers in the fermentation industries. They produce the disagreeable bitterness, unpleasant flavors, persistent cloudiness, and other unfavorable products and conditions.

Occurrence and Functions.—Yeasts are widely distributed in

nature, occurring wherever there are sugars. Although soils are not their normal habitat, they occur there to a limited extent. Fruits, and especially the overripe nectar of flowers and saps on the outer part of trees, are especially rich in yeasts. These are distributed by insects, some of which constantly harbor yeasts in their alimentary canal.

Yeasts ferment sugars, with the production of alcohol and carbon dioxide; hence, they are of prime importance in the production of alcohol and as leavening agents. They are also used in the commercial production of glycerin from sugar. They are active in the silo and play a rôle in the ripening of sauerkraut and other pickled foods. They have been used to combat, with questionable success, certain bacterial infections such as boils and carbuncles. They are often taken for their laxative effects. Their vitamin B content is usually high and they are at times used to supply this vitamin: It should be remembered, however, that the vitamin content varies with the species and especially with the medium in which they were propagated. A few yeasts are pathogenic and produce disease in man and the lower animals.

MOLDS

If bread, overripe fruit, cheese, grain, and even paper are left in a dark, moist, warm room molds soon cover them. A careful examination shows the molds to be composed of a tangled mass of tiny threads which, collectively, are called the mycelium. Each thread, often branching, is a hypha. The hypha is composed of cylindrical chainlike cells strung end to end. Hyphae are of two classes: (1) Vegetative hyphae, which are like the roots of a plant and extend into the substrate and serve for the purpose of taking up of nutrients; and (2) fertile hyphae which like the body of the plant rise above the medium and bear the spores.

Molds are of two types: (1) *Septate,* in which the hyphae are divided at intervals by a cross wall, each compartment being a complete cell composed of protoplasm made up of ectoplasm, cytoplasm, and nucleus; and (2) *nonseptate,* in which the hyphae are not divided by a cell wall, the nuclei are distinct but the cytoplasm of one cell blends into that of the adjacent cell. These are sometimes considered as large cells containing numerous nuclei, but it is probably more correct to consider such hyphae as multicellular. Mold cells are similar to yeast cells in that they possess nuclei, cytoplasm, and cell wall. They

also frequently contain vacuoles, granules, and other cell inclusions.

Molds grow by an enlargement of individual cells or by an increase in the number of cells. Usually it is a terminal cell which divides, which is called apical growth. Occasionally cells within the hyphae may divide, followed by an increase in size; this is intercalary growth. The cell nucleus may divide followed by the appearance of a bud on the side of the hypha which gives rise to the branching effect occurring in some molds; consequently, there is a division of labor among the molds. They differ further from the bacteria and yeast in being multicellular.

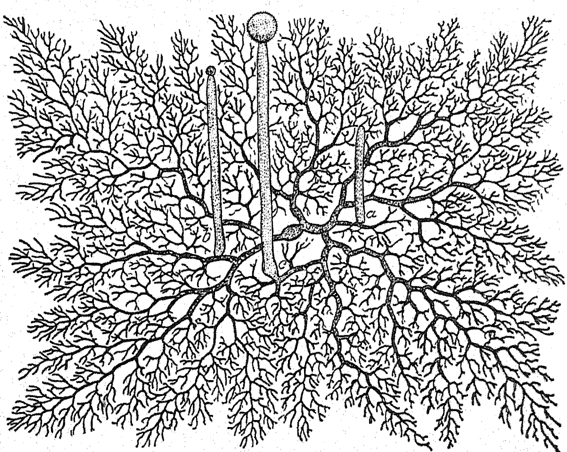


Fig. 37.—A mold (mucor mucedo) single-celled mycelium with three hyphae and one developing sporangium. (*After Kny, from Tavel.*)

Methods of Reproduction.—Molds multiply in various ways. In some species whole hyphae break up and each segment may be the starting point for a new colony. Molds usually reproduce by means of spores. These may be produced either asexually or sexually. Most molds bear asexual spores; their size, shape, color, and manner of production vary widely and are made use of in their classification. Asexual spores are borne either free or in a specialized spore sac called a sporangium; conidia are borne free at the ends of filaments of aerial mycelium. Sexual spores are of two types: (1) Zygospores, produced by molds possessing nonsegmented hyphae, and (2) ascospores, formed by molds having septate hyphae. In both cases,

cells of two adjoining hyphae fuse and give rise to the mother cell which produces the spores.

Mold spores differ from bacterial spores in being less resistant; and spore formation is a method of multiplication in addition to regeneration. Molds bear their spores on tiny upright hairlike threads; consequently, they are easily dislodged by air currents. The moldy odor often observed on entering damp, poorly lighted rooms is due to this property, as is also the wide distribution of molds. Mold spores are a resting stage in the life of the plant and hence are more resistant to adverse conditions, drying, heat, and chemicals than are the vegetative forms. They are considerably more resistant than the spores of yeasts but less resistant than those of bacteria.

Conditions for Mold Growth.—Molds grow more slowly than do bacteria; consequently, are not often found growing under conditions in which they have to compete with bacteria. Where conditions are inimical to bacteria, molds often flourish. They tolerate a high osmotic pressure and grow on jellies, preserves, and even on the brine solutions of pickling vats. They are especially troublesome on the surfaces of salted meats. Substances quite insoluble furnish a suitable substrate on which to grow. They will attack the leaves of a book, cotton or linen cloth, and even leather, provided there is sufficient moisture. Often alkali soils contain more molds than bacteria.

Molds tolerate a higher acidity than do bacteria, a factor which is used in their separation from bacteria. They show a special predilection for acid fruits and are often found growing in laboratory reagents. In acid soils they may predominate over bacteria.

Although molds are found in damp places they require less moisture than either yeasts or bacteria. Seeds, grains, and milling products may be moist enough to mold yet not sufficiently moist to permit bacterial decomposition. Molds are all aerobic and are often seen growing on the surface but not inside fruits and vegetables. Butter is wrapped and cheese paraffined primarily to prevent the growth of molds. If any air is left under the paper covering butter or if a slight crack appears in cheese, molds gain access and grow. They do not grow in hermetically sealed fruits and vegetables. Occasionally, there is seen in such products a bit of mold growth which entered before the container was sealed and grew until the free oxygen had been exhausted. They, like bacteria, are heat-loving and light-avoiding.

The optimum temperature varies with different molds but is approximately 30° C. They are all killed in a few minutes at the boiling point of water, but are somewhat more resistant to dry heat.

Importance of Molds.—Molds often contaminate laboratory cultures. They play a major rôle in the spoilage of fresh and dried fruits. They must be constantly guarded against in the storage of jellies, pickles, butter, cheese, and salted, dried, and smoked meats. They cause considerable trouble in the meat-packing industry, particularly with various kinds of preserved meats. A superficial mold growth appears on cured hams, sausages, and bacon which greatly detracts from their appearance. They occur in soils and play an important part in rendering plant food available and in the cycle of the elements.

Molds are used in the industries. Species of Penicillium play an important part in the ripening of Camembert and Roquefort cheese. Some molds produce highly active diastatic enzymes and are used in the preparation of alcohol from starches. They are also used for the production of organic compounds of commercial importance, some of which will be considered later. Few molds are pathogenic, but they produce disease in man and the lower animals to a much greater extent than do yeasts.

Three diseases of man due to molds are: Ringworm, favus, and thrush. Many molds have been described as plant pathogens and others may be a source of danger to man on account of toxic properties. According to Jordan: "Ergotism or ergot poisoning, often called in the Middle Ages saint's fire or the fire of Saint Anthony, is caused by the use of rye that has become diseased through the attack of a fungus, *Claviceps purpurea*. It has occurred frequently in the past when in times of famine the ergot or spurred rye was used in default of better food. It is said that 40,000 persons perished from this cause in Limoges in 922. Modern improvements in the facilities for transporting food from regions of abundant harvest into regions where crops have failed, and the use of special methods for separating the diseased grain from the wholesome have greatly reduced the prevalence of ergotism."

ACTINOMYCES

A widely distributed, important, and interesting group of micro-organisms are the actinomyces, or ray fungi. They are moldlike, consisting of extremely fine mycelia composed of hy-

phae which exhibit true branching, like the higher fungi. They possess both vegetative and reproductive hyphae. The mycelium is apparently nonsegmented and devoid of a definite nucleus. They reproduce by fragmentation of the mycelium and the production of conidia. In fragmentation, the aerial hyphae break into short fragments resembling in shape and in their protoplasmic properties, bacterial rods. Each of these can grow into complete fungi. The reproductive conidia are produced by a simultaneous division of the protoplasm in the sporogenous hyphae, progressing from the tip to the base. The conidia are more resistant than the vegetative hyphae. They vary in color from white to black. The colonies are often brilliantly colored. Most species emit a characteristic earthy odor.

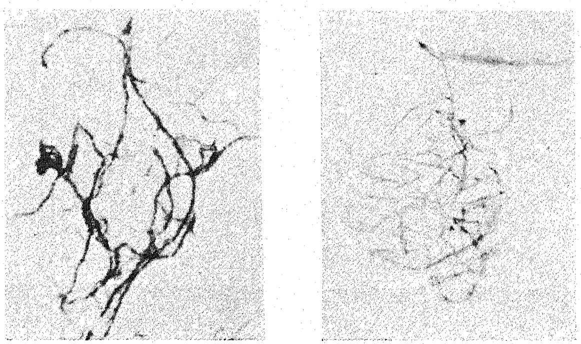


Fig. 38.—Soil actinomyces.

Actinomyces are closely related to both molds and bacteria, as is manifested by the theories offered concerning their origin: (1) That they are a higher development of the bacteria, (2) that they are degraded molds, and (3) that they represent a common ancestral stock from which both bacteria and mold have developed. Waksman indicates this relationship diagrammatically as follows:

Yeasts
↗
Actinomyces ——→ mycelial forms ——→ Oidium ——→ Hyphomycetes
↘
bacillary form → Mycobacteria → Corynibacteria → Bacteria

The molds are more resistant to acids than are bacteria, whereas actinomyces are more sensitive. Some of them have much in common with the tubercle bacilli in that they are acid-fast and will give rise to a disease of the lungs similar to tuberculosis.

Actinomyces bovis gives rise to lumpy jaw in cattle, which is characterized by a hard nonpainful lump on the side of the jaw. This develops a fistula from which pus drains, containing the ray fungi. They may also affect other parts of the body, and if the udder is infected they pass into the milk and may give rise to disease in man. One species *Actinomyces scabies* is considered to be the cause of potato scab and probably of other root crops. The saprophytic forms occur in large numbers in the soil and play an active part in the decomposition of both nitrogenous and non-nitrogenous organic matter. They are among the most common forms met with in the laboratory and, like molds, are a constant source of irritation to the bacteriologist on account of the frequency with which they contaminate his cultures.

References

Guilliermond, A., Tanner, F. W.: The Yeast. John Wiley and Sons, New York, 1920.

Henrici, A. T.: Molds, Yeasts, and Actinomycetes. John Wiley and Sons, New York, 1930.

Klocker, A.: Fermentation Organisms. Longmans, Green and Company, New York, 1903.

Thom, C.: The Penicillia. The Williams and Wilkins Company, Baltimore, 1929.

Topley, W. W. C., and Wilson, G. S.: The Principles of Bacteriology and Immunity. William Wood and Company, New York, 1932.

CHAPTER VIII

CLASSIFICATION OF BACTERIA

Organization is essential to life. The lack of it is death. Classification is a primary requisite to efficiency and progress in human activity, and as soon as it is absent the result is chaos. Even in the infancy of the race man endeavored to organize and classify the things with which he dealt. In most forms of modern endeavor the materials with which man deals are so numerous and complex that organization and classification become not only valuable but absolutely essential to progress. The astronomer classifies the stars, and the chemist the numerous compounds with which he works. Likewise, the biologist must classify the plants and animals with which he works.

Functions of Classification.—With the aid of a careful system of classification the librarian can quickly locate any volume in the library. By a different system the police officer is at once able to tell whether the finger prints before him are those of one of the millions of prints which are on file in the country. Thus, the aim of classification in any science is to group things in such a way as to bring out their similarities and differences. All individuals in the world could be classified according to finger prints. First, they could be divided into five great divisions according to finger-print patterns—arches, loops, groups, whorls, and central pockets. Those belonging to the arches could then be divided into seven classes—natural, dotted, tented, approximating loops, transitional patterns, staircase arches, and irregular arches; and each of these in turn could be further subdivided until even with such a complex aggregate as the finger prints of humanity a few very similar ones could be placed together, and what at first appeared a bewildering mass of data becomes an organized workable whole. The function of classification then may be summarized according to Huxley's definition as modified by Jevon as follows:

"By the classification of any series of objects is meant the actual or ideal arrangement together of those things which are alike and the separation of those things which are unlike, the purpose of the arrangement being primarily, to disclose the correlations or laws of union of properties and circumstances and secondarily, to facilitate the operations of the mind in clearly conceiving and retaining in memory the characters of the objects in question."

Method of Classification.—Classification brings out relationships. This is accomplished by arranging groups within groups. It is used in all our political and religious organizations, but probably reaches its highest state of perfection in the army. An army is a specific organized system which is composed of definite organized units, each unit of which is nicely fitted into the whole. That is, an army is composed of divisions; each division is composed of brigades; each brigade of regiments; each regiment of battalions; each battalion of companies; each company of platoons; and each platoon of squads. In such an organization each individual occupies a definite specific place. A somewhat similar arrangement is used in classifying the 250,000 species of plants which have been studied. We have seen that the plant kingdom is divided into four great divisions. Each of these divisions is composed of classes; each class is composed of orders; each order is composed of families; each family is composed of tribes; each tribe is composed of genera; and each genus is composed of species. Within the species there may be different races or strains. In a system such as this each plant has a specific place in the classification.

The division in which we are interested, the Thallophytes, is composed of two classes: (1) The algae which possess the green pigment, chlorophyll, and are, therefore, able to manufacture their own food. They include all the seaweeds and their fresh-water allies. (2) The fungi which lack chlorophyll and, consequently, are obliged to obtain their food from living animals and plants, or from dead organic material. To this group belongs that vast array of organisms—the bacteria, molds, yeasts, blights, rusts, toadstools, mushrooms, and similar plants. The order Eubacteriales, or true bacteria, one of the subdivisions of the general class is divided into families, these into tribes, and so on. But before following these subdivisions further it is well to consider briefly the factors used in the classification of bacteria and the method of naming them.

Nomenclature.—In the early history of botany the worker, in referring to a species of plant, used a descriptive phrase. Another worker might use a different phrase to describe the same plant. This resulted in confusion, for it was often impossible to determine whether both writers were referring to the same or to different plants. To overcome this difficulty the great Swedish naturalist, Linnaeus, devised the Binomial System of naming plants, so called because each species is given two names. The first is the name of the genus and the second the name of the species. The first is common to many; the second name is specific. The method is similar to that used in naming individuals. The surname is common to the family; the given name is specific. However, it will be noted in botanical nomenclature that the specific name comes last, or, in other words, it is the reverse of what it is in family names. Therefore in botanical work one gives first the name of the genus which must be capitalized and which may be written in full or abbreviated. This is followed by the specific name of the species. The name of the species is written in full and with a small letter. Usage at times sanctions the use of a capital where the name is a proper noun. However, even then it is better to use a small letter. In order to avoid confusion and to make more clear what specific plant is referred to there is often placed after the name of the species the name or abbreviation of the name of the botanist who first used the name for the species. Using this system, the name for wheat would be written *Triticum sativum* or *T. sativum*. That for the potato is written *Solanum tuberosum* or *S. tuberosum*. Unfortunately, this system is applied less satisfactorily with regard the micro-organisms.

Difficulties in Classifying Bacteria.—The difficulties inherent in the classification of bacteria are numerous. (1) The small, simple structure of the micro-organisms makes it impossible to work out a satisfactory classification on a purely morphological basis as in the case of the higher plants. (2) Many physiologic characteristics, such as pigment production which at first sight may appear useful, are not constant and, hence, cannot be used. (3) Our knowledge of the characters and make-up of the bacteria even at the present time is far from complete. (4) Bacteria play a part in many fields of activity, and consequently the criteria whereby they are recognized vary greatly according to the art or science in which they are studied. For agricultural and industrial purposes it may be more practical and quite

sufficient to classify bacteria according to their activity. For example, we may classify them as butyric- or lactic-acid-producing, ammonifying, nitrifying, or nitrogen-fixing organisms. (5) There has been a great tendency on the part of many authors to apply whole descriptions to micro-organisms instead of using the binomial names as agreed to in botany. For instance, the following cumbersome unscientific designation has been used: *Micrococcus acidi paralactici liquefaciens Halensi* or *Granulobacillus saccharobutyrecus immobilis liquefaciens*.

Classification of Bacteria.—Numerous attempts have been made to classify bacteria but none is without flaws. The one most extensively used throughout America in the past was the one proposed about thirty-five years ago by Migula. Migula makes use of morphology, and groups bacteria according to method of multiplication and motility.

This classification served a useful purpose for some time and had an important influence on bacterial nomenclature and grouping. However, it is especially unsatisfactory for agricultural bacteriology as it is hard to place some of the soil organisms and plant pathogens in the classification. The small number of genera makes it necessary to classify together organisms which are morphologically similar but which possess marked differences physiologically. Many attempts have been made to devise a classification which will overcome these difficulties. In 1909 Orla Jensen, a Danish bacteriologist, proposed a system in which biochemical properties were used. He coined names suggestive of the activity of the micro-organism. Recently a committee of the Society of American Bacteriologists formulated a method in which both the morphology and physiology of the organisms are made use of in their classification. Moreover, the method of naming is that followed by the botanists. The important points can be summarized as follows: (1) The name should be a binomial and not a trinomial. (2) Latin names should be used for all groups, and with certain limitations the oldest designation for each organism is to be preferred. (3) In naming, orders are designated with the ending *ales*, families with the ending *aceae*, subfamilies with *oidiae*, tribes with *eae*, and subtribes with *inae*. (4) The generic name is written with a capital. Specific names begin with small letters.

The families and genera of the order Eubacteriales, as reported by the committee, are as follows:

Class Schizomycetes. Simple and undifferentiated forms, without true branching.

Order Eubacteriales. Simple and undifferentiated forms, the true bacteria.

FAMILY I. Nitrobacteriaceae: Organisms obligate aerobes, using oxygen for direct oxidation of carbon, hydrogen, sulphur or nitrogen or compounds of these. Cells usually rod shaped, occasionally spherical.

Tribe I. Nitrobacterieae organisms oxidize simple inorganic compounds of carbon, hydrogen, sulphur, or nitrogen, or oxidize ethyl alcohol to acetic acid.

GENUS I. Hydrogenomonas cells capable of securing growth energy by the oxidation of hydrogen to form water.

GENUS II. Methanomonas cells oxidize methane to form CO_2 and water.

GENUS III. Carboxydomonas cells oxidize CO to form CO_2.

GENUS IV. Nitrosomonas cells oxidize ammonia to form nitrites. Rod shaped.

GENUS V. Nitrosococcus cells oxidize ammonia to nitrites. Large spherical organisms.

GENUS VI. Nitrobacter cells oxidize nitrites to nitrates.

GENUS VII. Acetobacter cells oxidize alcohol to form acetic acid.

GENUS VIII. Thiobacillus cells oxidize compounds of sulphur.

Tribe II. Azotobacterieae organisms capable of fixing free nitrogen of the air.

GENUS IX. Azotobacter cells capable of fixing free atmospheric nitrogen when grown in solutions of carbohydrates.

GENUS X. Rhizobium cells capable of fixing free nitrogen when growing symbiotically on the roots of Leguminosae.

FAMILY II. Coccaceae cells spherical. Metabolism complex, usually involving the utilization of amino-acids or carbohydrates.

Tribe I. Streptococceae parasites (except Leuconostoc) growing best in media containing serum. Occur in pairs or chains.

GENUS I. Diplococcus parasites growing poorly, or not at all, on artificial media. Cells usually in pairs.

GENUS II. Streptococcus chiefly parasites. Normally forming short or long chains, sometimes pairs, but never packets.

GENUS III. Leuconostoc saprophytes, usually growing in cane sugar solutions, and fruit juices. Cells in pairs or chains.

Tribe II. Neisserieae strict parasites, cells normally in pairs.

GENUS IV. Neisseria gram-negative organisms. Occur usually as pairs, occasionally as tetrads.

GENUS V. Graffkya gram-positive organisms occurring in tetrads, pairs and irregular masses.

Tribe III. Micrococceae. Facultative parasites or saprophytes. Cells aggregates of groups, packets or zoogleal masses. Growth abundant.

GENUS VI. Staphylococcus usually parasitic. Cells occur singly, in pairs, and in irregular groups dividing in one or two planes at right angles to each other or in irregular planes.

GENUS VII. Micrococcus. Facultative parasites or saprophytes. Cells in plates or in irregular masses (never in long chains or in packets).

GENUS VIII. Sarcina, cell division occurs in three planes forming packets.

GENUS IX. Rhodococcus, saprophytes. Cells in groups or packets. Form red pigment on agar.

FAMILY III. Spirillaceae. Cells spiral.

GENUS I. Vibrio, cells short, bent rods, rigid, single or united into spirals.

GENUS II. Spirillum. Cells rigid, of varying thickness and length and pitch of the spiral, forming either long curves or portions of a turn.

FAMILY IV. Bacteriaceae cells straight rods. Not producing endospores.

Tribe I. Chromobacterieae. Produce pigment on solid media. The pigment may be red, yellow, violet, green, or blue.

GENUS I. Serratia, small, aerobic rods, producing a red or pink pigment on agar or gelatin.

GENUS II. Flavobacterium, small aerobic rods, producing a yellow pigment on agar or gelatin.

GENUS III. Chromobacterium small, aerobic rods, producing a violet pigment on solid media.

GENUS IV. Pseudomonas, small, aerobic rods, producing a green or blue-green pigment.

Tribe II. Protominobacterieae. Nonpigment forming or producing red or yellow pigment. Capable of attacking the lower alkylamines.

GENUS V. One genus only.

Tribe III. Cellulomonadeae, cellulose decomposing organisms occurring in soil.

GENUS VI. Cellulomonas. One genus only.

Tribe IV. Achromobacterieae. Without pigment formation on agar or gelatin.

GENUS VII. Achromobacter. One genus only.

Tribe V. Erwineae. Plant pathogens.

GENUS VIII. Erwinia. Motile rods. Flagella peritrichous.

GENUS IX. Phytomonas. Rods motile or nonmotile. Motile forms possess polar flagella.

Tribe VI. Lactobacilleae rods often long and slender. Gram-positive. Nonmotile. Usually producing lactic acid from carbohydrates.

GENUS X. Lactobacillus. One genus only.

Tribe VII. Propionibacterieae. Nonmotile gram-positive rods. Ferment lactic acid, carbohydrates and polyalcohols with the formation of propionic and acetic acid and CO_2.

GENUS XI. Propionibacterium. One genus only.

Tribe VIII. Kurthieae. Gram-positive rods, growing freely on artificial media. Do not attack carbohydrates.

GENUS XII. Kurthia. One genus only.

Tribe IX. Pasteurelleae. Gram-negative rods, showing bipolar staining. Parasitic forms.

GENUS XIII. Pasteurella. One genus only.

Tribe X. Klebsielleae. Encapsulated, gram-negative, nonmotile rods.

GENUS XIV. Klebsiella, one genus only.

Tribe XI. Hemophileae minute parasitic forms growing only in the presence of hemoglobin, ascitic fluid or other body fluids.

GENUS XV. Hemophilus. Aerobic species.
GENUS XVI. Dialister. Anaerobic species.

Tribe XII. Bacterieae. Gram-negative rods growing freely on artificial media. Generally act on carbohydrates with formation of acid and gas.

GENUS XVII. Escherichia. Gas formed from dextrose and lactose. Acetylmethylcarbinol not formed from dextrose.
GENUS XVIII. Aerobacter. Gas formed from dextrose and lactose. Acetylmethylcarbinol formed from dextrose.
GENUS XIX. Proteus. Gas formed from dextrose and sucrose but not from lactose.
GENUS XX. Salmonella. Gas formed from dextrose but not from sucrose or lactose.
GENUS XXI. Eberthella. Forms acid but not gas from dextrose. Motile.
GENUS XXII. Shigella. Forms acid but not gas from dextrose. Nonmotile.
GENUS XXIII. Alcaligenes. Does not form acid or gas from any of the carbohydrates.

Tribe XIII. Bacteroideae. Motile or nonmotile rods, without endospores. Obligate anaerobes.

GENUS XXIV. Bacteroides. Only one genus.

FAMILY V. Bacillaceae—rods producing endospores, usually gram-positive. Flagella, when present, generally peritrichous.

GENUS I. Bacillus aerobic forms, mostly saprophytes.
GENUS II. Clostridium, anaerobic forms, often parasites.

An outline of the orders, families, tribes and genera is given below. Those wishing more complete information are referred to Bergey's Manual of Determinative Bacteriology.

CLASS SCHIZOMYCETES

Order	Family	Tribe	Genus
Eubacteriales	Nitrobacteriaceae	Nitrobacterieae	Hydrogenomonas
			Methanomonas
			Carboxydomonas
			Nitrosomonas
			Nitrobacter
			Nitrosococcus
			Acetobacter
			Thiobacillus
		Azotobacterieae	Azotobacter
			Rhizobium
	Coccaceae	Streptococceae	Diplococcus
			Streptococcus
			Leuconostoc
		Neisserieae	Neisseria
			Gaffkya
		Micrococceae	Staphylococcus
			Micrococcus
			Sarcina
			Rhodococcus
	Spirillaceae		Vibrio
			Spirillum
	Bacteriaceae	Chromobacterieae	Serratia
			Flavobacterium
			Chromobacterium
			Pseudomonas
		Cellulomonadeae	Cellulomonas
		Achromobacterieae	Achromobacter
		Erwinieae	Erwinia
			Phytomonas
		Lactobacilleae	Lactobacillus
		Propionibacterieae	Propionibacterium
		Kurthieae	Kurthia
		Bacterieae	Escherichia
			Aerobacter
			Proteus
			Salmonella
			Eberthella
			Shigella
			Alcaligines
		Pasteurelleae	Pasteurella
		Klebsielleae	Klebsiella
		Hemophileae	Hemophilus
			Dialister
		Bacteroideae	Bacteroides
	Bacillaceae		Bacillus
			Clostridium
Actinomycetales	Actinomycetaceae		Actinobacillus
			Leptothricia
			Actinomyces
			Erysipelothrix
	Mycobacteriaceae		Mycobacterium
			Corynebacterium
			Fusiformis
			Pfeifferella

CLASS SCHIZOMYCETES (*Continued*)

Order	Family	Subfamily	Tribe	Genus
Chlamydobacteriales	Chlamydobacteriaceae			Leptothrix
				Didymohelix
				Crenothrix
				Sphaerotilus
				Clonothrix
Thiobacteriales	Rhodobacteriaceae	Chromatoideae	Thiocapseae	Thiocystis
				Thiosphaera
				Thiosphaerion
				Thipcapsa
				Thiosarcina
			Lamprocysteae	Lamprocystis
			Thiopedieae	Thiopedia
				Thioderma
				Lampropedia
			Amoebobactereae	Amoebabacter
				Thiodictyon
				Thiotheca
				Thiopolycoccus
			Chromatieae	Chromatium
				Rhabdomonas
				Thiospirillum
				Rhodocapsa
				Rhodotheca
		Rhodobacteroideae		Rhodocystis
				Rhodonostoc
				Rhodorhagus
				Rhodobacterium
				Rhodobacillus
				Rhodovibrio
				Rhodospirillum
	Beggiatoaceae			Thiothrix
				Beggiatoa
				Thioploca
	Achromatiaceae			Achromatium
				Thiophysa
				Thiospira
				Hillhousia
Myxobacteriales	Myxobacteriaceae			Myxococcus
				Polyangium
				Chondromyces
Spirochaetales	Spirochaetaceae			Spirochaeta
				Saprospira
				Cristispira
				Borrelia
				Treponema
				Leptospira

CHAPTER IX

BACTERIAL VARIATION

PROBABLY few subjects in the whole realm of biology have had a more checkered career than the subject of bacterial variation. The great variety of micro-organisms, their minute size, their apparently simple structure and rapid multiplication, and their growth in various media early led to the view that they were extremely variable. Nägeli taught that there was a rapid and almost unlimited variation in the characters of a single species, whereas Cohn and Koch took the stand that individual species preserve their morphological and physiologic characteristics with great constancy. Where variants were observed, too often they were dismissed as contaminants. It was admitted that old cultures and those grown under abnormal conditions often varied greatly, but these were called "involution forms" which may be prevented by constancy of media, temperature, age, and the like. Hence, it became the practice to study micro-organisms under constant conditions. Nevertheless, variants like smoldering embers occasionally appeared, and within recent years these embers have been fanned into a flame which is recognized but variously interpreted by modern bacteriologists, as is evident by the multiplicity of terms which have been applied to the phenomenon.

Temporary Variation.—Variation may be of two kinds, temporary and hereditary. Temporary changes or modifications are usually due to environmental conditions and are not passed down from generation to generation. Some bacilli may reproduce club-shaped organisms many times larger than the average. This is characteristic of the bacteria which cause diphtheria, whereas the bacteria which grow in the nodules on the roots of alfalfa often take on the forms of stars, crosses, and various grotesque shapes. These have been called "involution forms" of bacteria and have been likened to the lame and halt in human society and called degenerate forms of bacteria. This, however, is hardly an apt illustration or a correct designation, for these peculiar shaped bacteria which possess all powers of normal bacteria, and if they find their way into the body of

an animal they are just as likely to produce their disease as are the normal micro-organisms.

Hence, we consider their changed shape as due to age or environmental conditions which have caused morphological changes but which have not affected their other properties and characteristics in the least. The cholera vibrio assumes in old cultures the most bizarre shapes, swollen spheres, crescents, half moons,

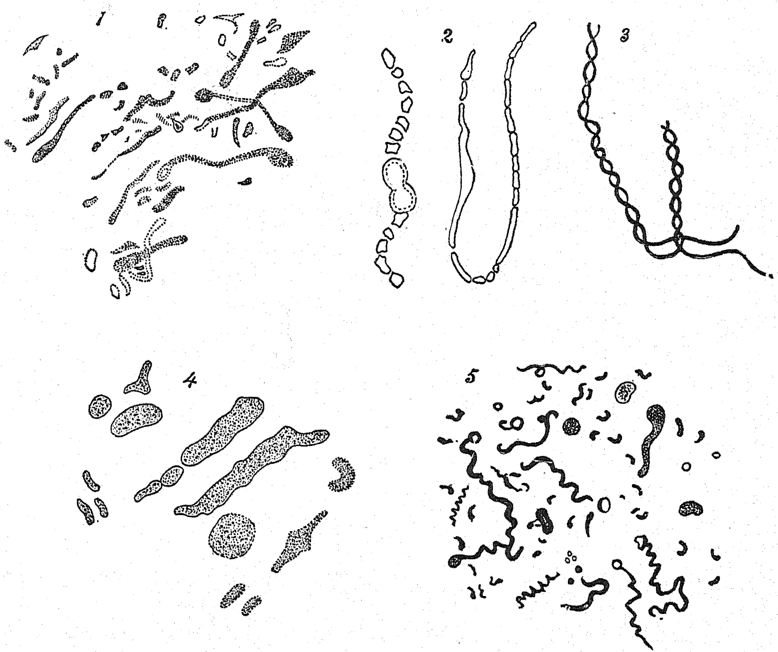


Fig. 39.—Involution forms of bacteria (enlarged about 1000). 1, *Proteus mirabilis.* (*Hauser.*) 2, *Aerobacter aerogenes.* (*Hauser.*) 3, Spirilla form of Bacillus anthracis. (*Petruschky.*) 4, Involution forms of *Bacterium halophilus.* (*Russell.*) 5, *Vibrio comma.* (*van Ermengem.*)

and distorted cells with knoblike protuberances. Similar changes occur when organisms are cultured on acid media; if transferred to media of proper alkalinity the type form appears. At times organisms are grown so as to bring out these distorted forms for diagnostic purposes. Today many bacteriologists consider "involution forms" of bacteria as representing stages in an orderly development toward old age. Few consider them as stages in a complex life cycle through which bacteria pass.

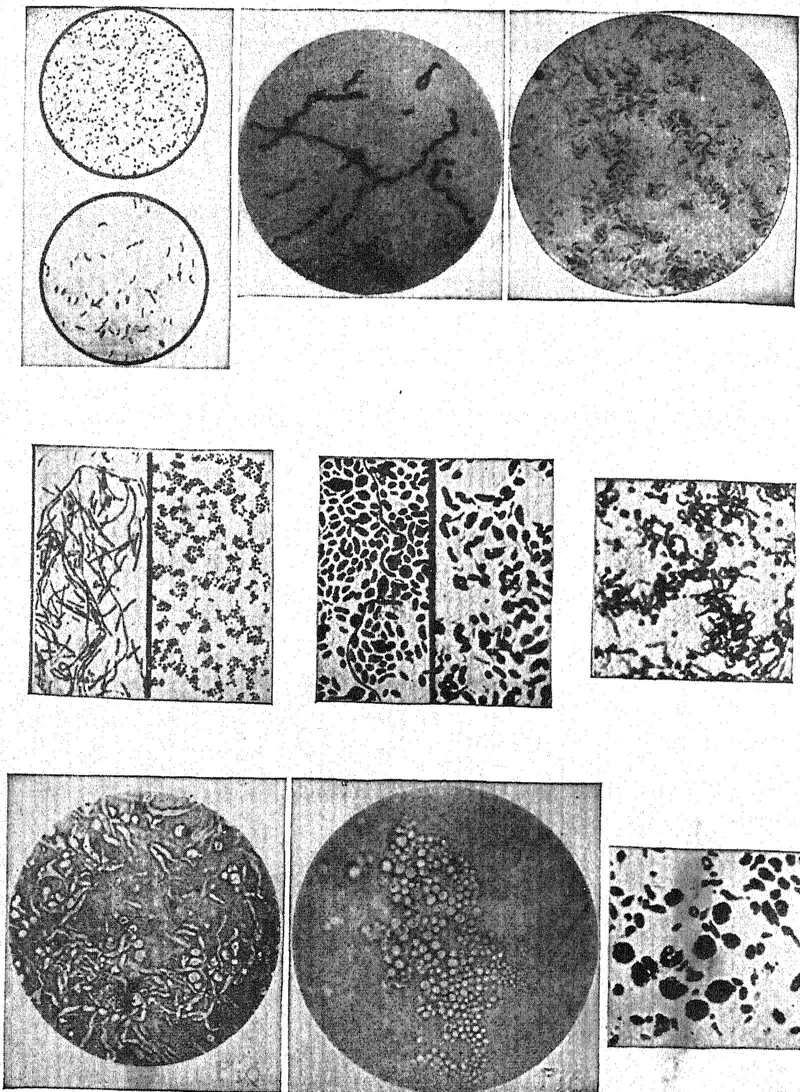


Fig. 40.—Cholera organism under various conditions of growth. (*After Henrici.*)

Physiologic properties may also change. Pigment-producing bacteria temporarily may lose their ability to produce pigments; carbohydrate-fermenting organisms may lose their ability to ferment carbohydrates. Various temporary changes in the growth of organisms in laboratory media may also appear.

Hereditary Variation.—Hereditary variation occurs more quickly and is transmitted to the progeny. They are often referred to as "mutants." A mutation, according to Dobell, may be defined as "any permanent change which is transmitted to subsequent generations of bacteria, without implication regarding the suddenness or gradualness of the change, or the manner of its acquisition." This is well exemplified in a change desig-

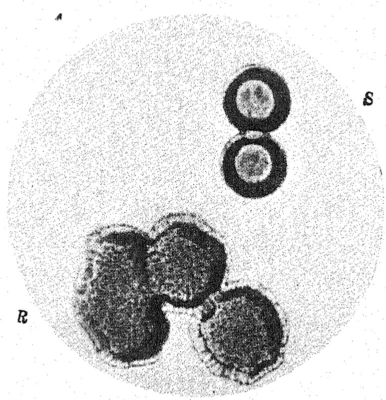


Fig. 41.—Smooth (*S*) and rough (*R*) colonies of *Eberthella typhosa*. (*After Arkwright.*)

nated as "bacterial dissociation." The colonies produced by the coli-typhoid-dysentery group, together with those of many other bacteria on solid media, are rather moist and glistening with round edges and smooth surfaces; if grown under unfavorable conditions, there may appear spontaneously rough granular colonies. There may also appear a change in cell division. In smooth strains of *Salmonella aertrycke* growing in a thin layer of agar, each cell division is soon followed by a separation of the daughter cells which slip past each other and come to lie side by side. In the rough variant the daughter cells tend to adhere end-to-end for some time after dividing. Bacteria which normally produce rough colonies have been induced to produce smooth colonies, although this change is much less common. The

resulting rough and smooth colonies tend to breed true, the rough being the more stable. The smooth form is usually more virulent and is found to predominate in early typical cases of diphtheria. In atypical cases and in the carrier, the rough form predominates.

Weil and Felix found the normal form of Proteus $\times$ 19 to be motile and to produce characteristic spreading growth on agar. This they designated as the H form (German, Hauch = film). A nonmotile variant producing isolated colonies they designated as O form (German, ohne Hauch = without film). Similar H and O forms of many species of motile bacteria have since been studied, and the abbreviations "H" and "O" have taken their place in the bacteriological shorthand along with "R" (rough) and "S" (smooth) to denote particular types of bacterial variation.

In motile organisms it is usually the S form that is motile and in the pigment-producing organisms the S form is more likely to retain this property. The S type is more likely to produce capsules and its biochemical properties are more pronounced than in the R form. Of the pathogens, the S and H types are more virulent and best suited to the production of vaccines.

Do these discoveries point to transmutation of bacterial species? Some writers have seriously considered the possibility. According to Topley and Wilson, however, some bacterial types, which have been given specific rank, may have to be relegated to the position of variants as the result of closer study; this is altogether possible. As regards these species which have been most thoroughly and carefully investigated, however, the accumulation of evidence has steadily decreased the probability of the occurrence of any transmutation from one to another.

Great changes have been observed in a number of pathogens. Griffith claims that one type, pneumococci, may be transformed into another and Rosenow and co-workers claim to have been able to transmute streptococci into pneumococci. However, few bacteriologists are ready to accept this latter work as conclusive. If transmutation does occur, it is possible for new diseases to appear, and it is even claimed that some of the infections of the nervous system, such as sleeping sickness and infantile paralysis, are new diseases. The more common diseases, such as smallpox, tuberculosis, and anthrax, are as old as, and perhaps older than, the human race, yet at the present time they appear the same as in the past, fluctuating in virulence much as they do today.

Epidemics appear in cycles, and there are times when they have little tendency to spread and may have low mortality, whereas at other times they may spread like a prairie fire, sweeping all nonimmunes before them. There is some evidence substantiating the theory that this rise and fall of epidemics and variation in severity may be associated with bacterial dissociation.

CHAPTER X

STUDYING BACTERIA

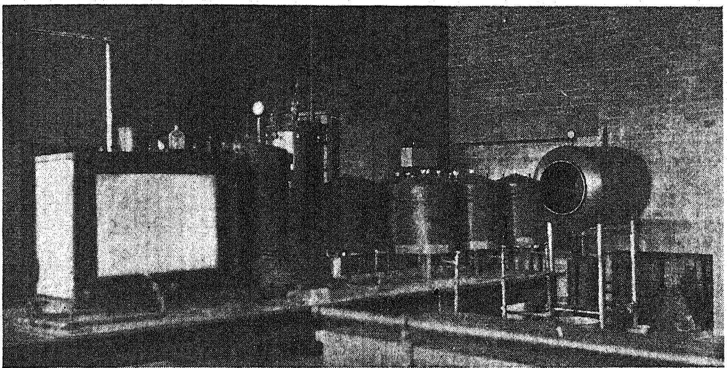
BACTERIA are ubiquitous; they are extremely small; and many of them are very resistant to external influences. The early workers in the field of bacteriology did not possess this information; hence, when bacteria were first discovered, it was only natural to conclude that they were spontaneously produced. This idea was overthrown only when man learned how to free his materials from these minute specks of life and to keep them free from external life. It was not until he had devised means for growing these micro-organisms that he learned their properties and characteristics. Their minute structure rendered useless the methods used to study the higher forms of life; so, it was necessary to devise technic peculiar to the specific subject. For freeing his materials from bacteria, man soon learned to use heat and chemicals; to keep them free, cotton; to grow them at will, certain foods; and to study their shape and structure, he used the microscope. He learned their action by studying the changes produced in the living and dead materials into which he placed them.

Sterilization.—Materials free from living micro-organisms are said to be sterile. The process by which they are rendered free is called sterilization. It is absolutely requisite that the worker in bacteriology have quick, easy, and effective methods of sterilizing the things with which he deals; otherwise, it would be impossible for him to gain specific information concerning any micro-organism, as the ones which he would find in his medium would be not only those placed there by himself but all others which chanced to be in his materials. We have seen that it was this very principle which played such a tremendous part in the controversy on spontaneous generation.

Sterilization may be effected by either chemical or physical means. We shall consider here the physical processes, leaving the chemical methods for a later chapter. The physical methods in general use are heat, light, and filtration.

Sterilization by Heat.—The most general method of sterilization is by means of heat. It is cheap, effective, and readily applied. It is used in various ways, depending upon the nature of the substance to be sterilized. The following are the principal methods used by the bacteriologist: (1) Flame, (2) dry heat, (3) boiling water, (4) streaming steam, and (5) steam under pressure.

The Flame.—The platinum needle, tweezers, and various other instruments used by the bacteriologist in handling infected material can be quickly and effectively sterilized by flaming. In this, certain precautions are necessary: (1) All parts of the object infected must be heated to a temperature high enough to



a b c d

Fig. 42.—Sterilizers. (*a*) Hot air sterilizers; (*b*) upright autoclaves; (*c*) steam sterilizers; (*d*) horizontal autoclave.

destroy all forms of life. (2) Where the infected object is covered with moisture, care must be taken not to heat too quickly; otherwise, spattering may occur. This may scatter the infected material about, and if pathogens are present dire results may follow.

Valueless infected objects may be burned. The sputum of individuals ill with communicable diseases may be received in clean rags and then burned, thus preventing the infecting of others. Before the development of bacteriology this was the principal method employed in protecting man against infection. Today it has been replaced mainly by less destructive methods.

Hot Air.—Many materials are too valuable to burn; hence, one must turn to other effective but less destructive methods of

sterilization. Petri dishes, test tubes, and many other forms of glassware may be effectively sterilized by heating in an oven for one hour at a temperature of 170° C. This is the temperature at which cotton just begins to char. Its effectiveness depends upon the charring of the bodies of the microbes. Although the common kitchen oven can be effectively used for this purpose, there are many easily operated gas or electric hot-air sterilizers on the market.

Boiling Water.—Forceps, scissors, knives, hypodermic needles, and many other objects may be readily freed of infection by boiling for ten minutes in water containing 1 per cent of soda. This is also an effective method for cleaning milk bottles, buckets, and other utensils.

Sterilization by Streaming Steam.—Moist heat is more effective in destroying micro-organisms than is dry. This is due to a number of causes: (1) Moist heat is more penetrating. (2) Life is destroyed when the protein composing the body is coagulated, and the temperature at which coagulation occurs depends upon the moisture present. Dry proteins can be heated to the charring point without coagulation. (3) Hot steam penetrates the various substances being sterilized, and on condensing liberates great quantities of heat which in turn penetrate all of the material; hence, the favorite method of sterilization is by means of steam. This, as generated, is known as streaming or live steam. One of the best methods of generating it for laboratory use is by means of an Arnold steam sterilizer. This is provided with a false bottom so that the steam passes up through the materials to be sterilized, and as the steam condenses the water is returned to the receptacle to be reheated. It should be provided with an opening in the top into which a thermometer can be fitted, in order that the worker may be certain of the temperature. Test tubes should be placed in wire baskets; other materials should be loosely packed so the steam can penetrate all. The temperature (100° C.) reached by streaming steam at sea level is sufficient to kill the nonspore-bearing organisms in fifteen minutes. Some resistant spores will withstand this treatment for hours. To obviate this difficulty, either the discontinuous method or steam under pressure is used.

Discontinuous or Intermittent Sterilization.—Discontinuous heating as a means of sterilization was introduced in 1877 by Tyndall, who made the following remark concerning it: "Five minutes of discontinuous heating can accomplish more than five

hours' continuous heating." This method takes advantage of the fact that all bacteria, while in the vegetative stage, are quickly killed by live steam but are very resistant when in the resting or spore stage. The material to be sterilized is heated in steam or boiling water from fifteen to sixty minutes, depending upon the nature and size of the container. In sterilizing agar, time is counted from the point at which the agar melts. Large containers require longer for the heat to penetrate than do small ones. After the material has been heated the necessary length of time, it is set aside for twenty-four hours in a com-

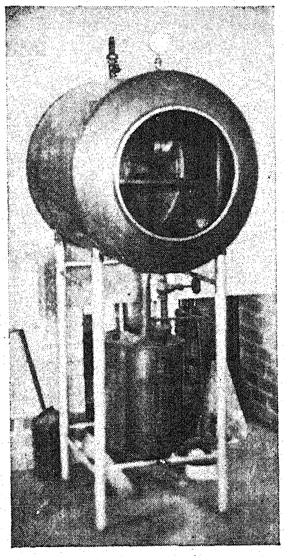


Fig. 43.—Autoclave.

paratively warm place. During this time the spores germinate. The material is then reheated, allowed to stand a second time, and after twenty-four hours again reheated. The process may be repeated a third or even a fourth time. This method is useful and effective for sterilizing media containing sugars or body fluids which would be changed by higher temperatures. It is often used by the housewife in the processing of fruits and vegetables. However, if the spores do not germinate between heatings it may fail. A failure of germination may be due to a number of causes: (1) The medium may be unsuited for the growth

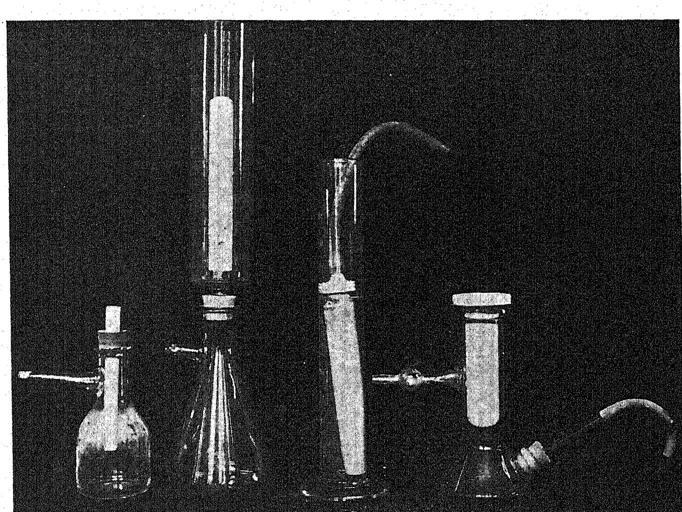
of some particular organisms present. (2) The material may have been overheated in the first process, and micro-organisms injured to such an extent that they will not germinate in the time allotted. (3) There may be present anaerobes which will not germinate in the media until the free oxygen has been removed. Such cases can be handled by heating to a higher temperature in steam under pressure. This is done in an autoclave.

Autoclave Sterilization.—Bacteria are killed by boiling water or live steam, but some spores resist these treatments for hours. Materials containing such spores may be sterilized by exposing for a time to a higher temperature. The temperature and time necessary depend on the nature of the material to be sterilized, as well as on the micro-organisms present. Agar media in test tubes are readily sterilized by exposing fifteen minutes to a temperature of 120° C. Larger containers require a longer time. The necessary temperature is readily obtained by heating in an autoclave. This is a cylindrical vessel in which the steam is generated or forced in at the required temperature. It is provided with a pet cock through which the steam may be permitted to escape, a pressure gauge, and often a thermometer. There is also a safety valve which can be set to slowly release the steam at any desired pressure. Certain precautions are necessary; otherwise, loss of media, injury to autoclave, or other accidents may result. (1) Care should always be taken to see that sufficient water is in the autoclave to carry it through the time it is being heated, for should it go dry it will be burned out and ruined. (2) Where test tubes in baskets are placed directly on top of each other there is a great tendency for the water to condense and run down into the under tubes, thus wetting the cotton stoppers and spoiling the media. (3) The steam should be allowed to issue from the opening several minutes before closing; otherwise, pockets of air are produced around the materials to be sterilized. This results in the pressure gauge giving a false reading in so far as temperature is concerned. (4) Time should be counted from the point at which the required pressure is reached. Agar should be in the liquid form when placed in the autoclave (or else a correspondingly longer time allowed for sterilization). (5) The pressure should be slowly raised to the desired point thus insuring proper heating of the material during sterilization. This is especially important where the autoclave is connected directly with the steam. (6) The temperature should be allowed to fall below the boiling point before

the autoclave is opened; otherwise, the plugs may be forced from the containers, or the contents themselves boil out.

Culture media (with the exception of those containing some of the carbohydrates or body fluids) may be sterilized by this means. Where gelatin is to be sterilized in the autoclave, the pressure should not exceed 10 pounds for five minutes, and the media quickly cooled in the ice box. If this is not done the gelatin will fail to solidify.

The autoclave is extensively used in the processing of various food products as well as in the sterilizing of infected bedding and clothing



a *b* *c* *d*

Fig. 44.—Different types of bacteriological filters: *a,* Kitasato; *b,* Berkefeld; *c,* Chamberland; *d,* Reichel. (*From McFarland Pathogenic Bacteria and Protozoa.*)

Sterilization by Filtration.—Air and other gases may be sterilized by drawing through cotton. Cultural media are readily preserved by stoppering the container with cotton and then sterilizing. However, if the stoppers become moist, or if considerable time elapses, certain micro-organisms tend to grow through the cotton plugs.

Various types of filters made of unglazed porcelain or compressed diatomaceous earth are used where the application of heat will injure the solution, as in the case of certain sugars,

tissue extracts, and serums. These filters are made in various grades of fineness; hence, they are sometimes used in separating organisms of varying sizes. Those forms of organisms which pass through the filter are spoken of as filtrable viruses. The filter itself and accompanying parts are at first sterilized in the autoclave, after which the material to be filtered is passed through. The filter can be cleaned by passing through it considerable clear water. Where the pores become clogged the filter can often be revived by heating; but in this case, care must be used to see that the heat has not produced small fissures which cause the filter to leak. One of the best methods of reviving a filter is to sterilize in an Arnold, gently brush the surface with a soft brush and then pass through it a 0.5 per cent solution of

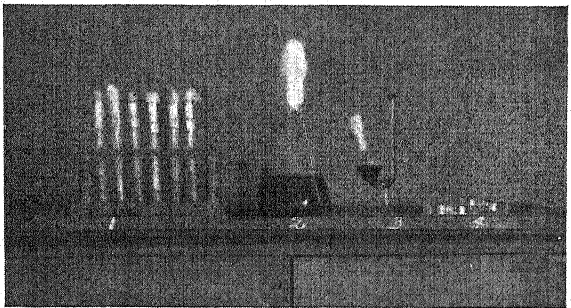


Fig. 45.—Apparatus used in the growth of bacteria. 1, Test tube cultures of bacteria; 2, Erlenmeyer flash of bouillon; 3, fermentation tubes containing bouillon; 4, Petri dish.

potassium permanganate followed by a 5 per cent solution of sodium bisulphite. This is washed out by passing through considerable quantities of distilled water.

Coarse filtration is sometimes accomplished by the use of paper, pulp, or sand. The latter is often used in filtering a city water supply.

Culture Media.—All nutrient materials used in the laboratory for the growth of micro-organisms are known as *cultural media.* They are essential for the keeping, growing, and the studying of the cultural characteristics of micro-organisms. They are variously classified as synthetic and nonsynthetic; liquid and solid; liquefiable and nonliquefiable.

Synthetic media are those composed of pure chemicals and of known composition. They are valuable in the work for which

they are used because they can be accurately duplicated by other workers. The goal toward which the bacteriologist strives is the preparation of synthetic media on which he can grow any of the micro-organisms. This is far from possible at the present time; hence, he uses nonsynthetic media, such as potatoes, milk, beef tea, blood, and other natural products.

A nutrient medium suitable for the growth of micro-organisms must possess the following characteristics: (1) It must contain nutrients suitable for the organism that one desires to grow. This may be a simple salt solution, as is used to grow some soil bacteria, or it may be a complex body tissue or fluid, as is the case with many of the pathogens. (2) It must possess a suitable reaction. Most bacteria require a neutral or slightly alkaline medium; whereas, many molds flourish in an acid medium. (3) The nutrient media must be sterile. The method used in sterilization depends upon the specific medium.

One of the principal liquid media is beef tea. When small quantities of the seaweed, agar-agar, are added to this, we obtain a solid medium. If gelatin is added in place of agar-agar, there results a medium which is solid but which can be liquefied by certain organisms. The nature of the growth and changes produced in the various media are used by the bacteriologist in the characterizing and identifying of bacterial species.

Cultures of Bacteria.—A culture is any growth of micro-organisms on laboratory media. A colony is a growth that has originated from a single organism. A culture in which only one species of organisms is present is called a pure culture; one in which more than one is present is mixed. As bacteria occur in nature, they are usually mixed. In order to study their specific properties, workers find it necessary to obtain them as pure cultures; hence, we shall consider very briefly the methods of obtaining pure cultures.

Pure Cultures.—Early workers used the so-called "dilution method" for obtaining pure cultures of micro-organisms. It consists in adding a small quantity of the material to be studied to a sterile liquid medium. A small quantity of this, in turn, is transferred to a second portion of sterile media, and this process is repeated a number of times. The inoculated medium is then incubated until growth appears on the more heavily seeded media. If the dilutions are continued far enough, some of the tubes will show no growth. Some of the tubes receiving only small quantities of the material may have received only a single

organism, and hence, these would be pure cultures. This, however, could be determined only by further microscopical and cultural studies. It is evident that such a method is based on the expectation that some tube is to receive only one species. Furthermore, the method is long and cumbersome and has been practically replaced by later and more specific methods.

Plate Method.—A great advancement was made in obtaining pure cultures when Koch introduced the use of solid media. In general, this method is as follows: Tubes of gelatin or agar

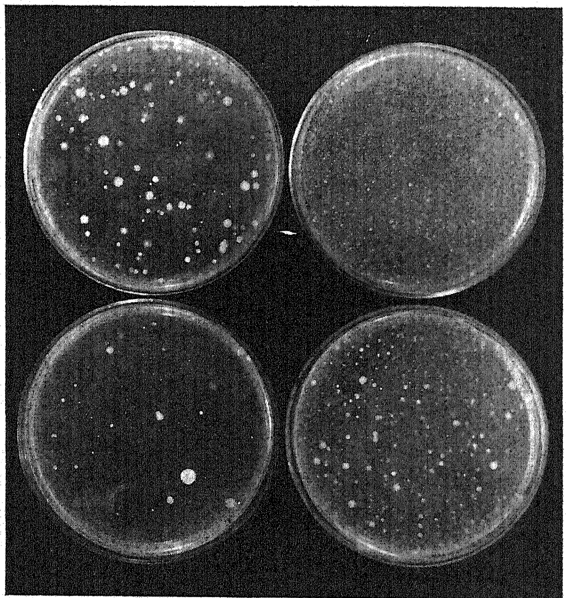


Fig. 46.—Dilutions made in Petri dishes.

media are liquefied and then cooled to 42° C. The material from which one wishes to obtain pure cultures is transferred to the liquefied tubes by means of sterile platinum or iridium loop known as an oese. A loopful of the material is placed into a tube of the medium and thoroughly mixed with the medium by tilting and rolling the tube, care being taken not to produce bubbles. From the well-mixed tube three loops are carried over to a second tube which is mixed in the same manner. From the second tube five loops are carried to a third tube and mixed. The lips of the tubes are flamed and the contents poured into

sterile Petri dishes. During the pouring the cover of the Petri dish is raised just sufficiently to permit pouring, the lid acting as a cover to prevent dust entering the plate. The dish is then agitated back and forth, so that the medium is evenly distributed over the surface. The plates are incubated at optimum temperature until colonies appear. It is well to invert agar plates while incubating, thus preventing the collection of moisture on the surface of the medium which would cause the colonies to run together.

When growth appears on the medium it is examined by the low-power of the microscope or a hand lens. Those colonies which stand out free from others and which have a characteristic homogeneous appearance are selected for further study. This is done by fishing out the isolated colonies with a platinum needle and studying them microscopically and culturally.

Microscopical Study.—Micro-organisms can seldom be identified by the microscope alone. It, however, will reveal certain characteristics, such as individual form, arrangement, reaction to stain, motility, or lack of motility, and often spore formation. Microscopical study may be made either on the living or killed stained bacteria.

The living organisms are most readily studied by the so-called "hanging-drop method." A small drop of the material to be examined is placed on a cover glass; this is fastened upon a hollow glass slide so that the drop hangs free from the center. This, examined under the microscope, will reveal the organisms especially at the edge of the drop; the cells are clearly visible and their motility or nonmotility can be readily determined.

Stained preparations are obtained for examination in the following manner: The bacteria are evenly distributed over the surface of a cover glass or glass slide and allowed to dry. These are fixed by quickly passing the slide, or cover glass through the flame, or covering it with special fixing agents. This causes the micro-organisms to adhere firmly to the surface of the glass. The surface is then flooded with a solution of one of the aniline dyes, and the glass is allowed to stand a short time, either in the cold or heated; depending upon the nature of the organism being examined. The slide then is washed in water (or, in some cases in alcohol or dilute acid), permitted to dry, and the organism on it examined microscopically. Where it is desired to preserve the specimen, the cover glass is fastened to the slide by means of a drop of Canada balsam.

For further study of the cultures, they are inoculated into various media and their growth characteristics studied. A descriptive chart, prepared and revised from time to time by a committee of the Society of American Bacteriologists, acts as a guide in this work.

Pure cultures of certain pathogens are obtained by inoculating them into susceptible animals. The body of the animal destroys the foreign organisms; whereas, the specific pathogen multiplies in definite organs from which it is later obtained in pure culture.

A few of the very resistant micro-organisms may be separated from less resistant species by means of heat or chemicals, but the details of this (as well as the above outlined methods) may be obtained by the student from the many available bacteriology laboratory guides.

CHAPTER XI

COMPOSITION OF BACTERIA

The child given a new toy proceeds at once to analyze it; for it is an inherent characteristic of the human race to ask of a thing, "Of what is it composed?" In the childhood of the individual, or the race, the answer is often incomplete and speculative, but within recent years man has progressed greatly in analyzing the earth on which he treads; the plants and animals with which he comes in contact; and he has reached out and

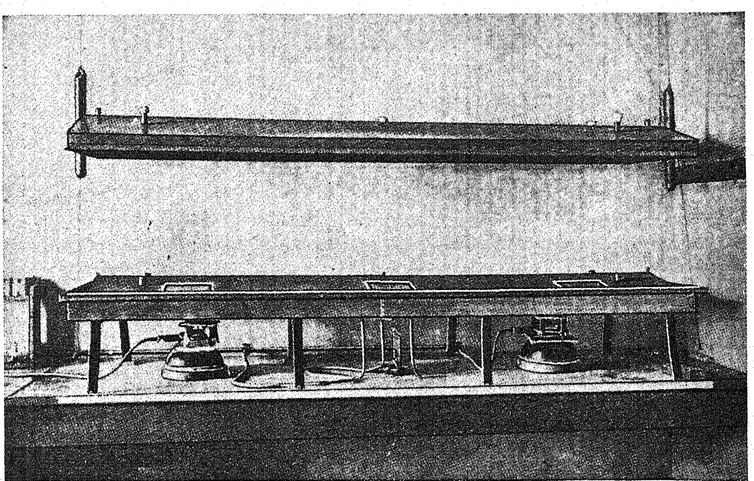


Fig. 47.—Vaughan and Novy's mass culture apparatus. Tanks with raised lid. (*After Vaughan.*)

analyzed the distant stars. Today, with a combination of chemical and microscopical means, he is analyzing not only the bodies of bacteria but also their individual parts.

How Accomplished.—Several methods have been used for obtaining bacteria for chemical analysis. The more important of these are the centrifugation and filtration of liquid cultures and the scraping of cultures from the surface of solid cultural

media. Vaughan obtained massive cultures by the use of large copper double tanks (10 feet long, 2 feet wide, and 4 inches deep) around the edge of which were troughs an inch deep into which were fitted the edges of covers. These tanks were partly filled with a nutrient agar, sterilized, and then inoculated with the desired organisms. The resulting profuse growth was scraped off, washed, and analyzed. The media was resterilized, and in this way several successive crops of the same organism were grown. However, it has been found that they do best when rotated, just as the scientific agriculturist has learned that he gets the best crops when he practices a system of crop rotation.

The analyst may determine (1) the elements which compose the bacterial cell, (2) the compounds composing it, (3) the composition of the various parts of the cell.

Elementary Composition.—One might expect to find in the body of the powerful little microbe the rare and costly elements, but in this, one is disappointed. Bacteria are composed of the common and more abundant elements—the same that compose the bodies of the higher plants and animals. Over 95 per cent of their bodies are made up of the four very common elements: Carbon, hydrogen, oxygen and nitrogen. The other 5 per cent consist of sodium, potassium, calcium, magnesium, iron, sulphur, phosphorus, and occasionally aluminum, silicon, manganese, copper, zinc, and at times still others. Bacteria are more like the animal than the plant in nitrogen content. Wheat contains approximately 2.5 per cent of nitrogen; soy beans, 5 per cent; cow's milk, 3.2 per cent; lean beef, 14 per cent; and the bacterial cells as much as 11 per cent, all calculated on the dry basis. Their phosphorus content is high when compared with that of other plants and animals. This one would expect, when it is recalled that their bodies are composed primarily of nuclear material, and nuclear material is especially rich in phosphorus. The composition of the wheat kernel varies with the variety, the water and the food received during the growing season; likewise, the composition of the bacterial cells varies with the species, age and the media on which they are grown.

Compounds Composing the Bacterial Body.—The compounds composing the bacterial body may be considered under the divisions of water and solids. The solids consist of carbohydrates, fats, proteins, ash, and certain less well-defined groups, as vitamins, pigments, toxins, and enzymes.

Water.—Bacteria consist principally of water. The quantity

present in the actively growing cell varies from 75 to 98 per cent. In general a particular kind of bacteria when grown on liquid medium contains more water than when grown on solid medium. The younger the culture, the more active the organism and the greater the quantity of water present. Old cultures contain considerably less, and when spores form the water content is greatly reduced. However, there is an irreducible minimum below which they cannot go and still maintain life. In fact, the absence of water means the absence of life, and next to living matter water is the most wonderful thing in the world. The ancient philosopher, Thales, considered water as the origin of all things. A little later, Empedocles and Aristotle added to this earth, air, and fire. These were the so-called "elements of the ancients." Of these, water, in many respects, is the most interesting; for all that is happening in man, plants, and the lower animals is intimately associated with the transformation of water.

Water is taken up by the roots of the plants; carbon dioxide is absorbed by the leaf, and under the influence of the active rays of light in the presence of chlorophyll there result sugars, starches, celluloses and the whole category of complex compounds found in plants. Each transformation (from a simple to a complex or from a complex to a simple compound) is accompanied by the combination, elimination, or production of water. The complex tissues of plants are taken into the bodies of animals, approximately two thirds of which is water. Here they are again caused to combine with water (or water is eliminated from them), and there result the mysterious tissues of the specific animal. Or if perchance the animal uses the plant tissues for energy, the end-products are principally water and carbon dioxide. The nutrients in plants and animals are carried into the body by water, and with water the waste products leave.

The automobile is kept at working temperature by encasing the cylinders in water and keeping it in rapid circulation. In a like manner, the living organism keeps its temperature normal. The water not only circulates, but it evaporates; hence, its efficiency is increased fifty-fold. Therefore, it is in water and with water that living tissues are dealing in all their complex processes of life.

Solids.—Solids comprise from 2 to 25 per cent of the bacterial cell. These consist of from 3 to 30 per cent of ash and from 70 to 97 per cent of organic material. The latter contains be-

tween 40 and 70 per cent of protein, 10 and 30 per cent of carbohydrates and 1 and 10 per cent of lipids. There may be also small quantities of toxins, pigments, and possibly vitamins.

Proteins.—Proteins are complex organic compounds composed of carbon, hydrogen, oxygen, nitrogen and usually sulphur. In addition to these, they sometimes contain other elements, especially phosphorus. When treated with strong acids or digested by animals or bacteria, they yield certain characteristic compounds called amino-acids. The so-called "simple proteins" yield only amino-acids, whereas the complex proteins yield amino-acids plus other compounds. Sometimes these are carbohydrates or fats. Others yield amino-acids plus purine bases. These are nucleoproteins. The bulk of the dry matter of the bacterial cell consists of protein. In this respect, the bacteria are more like animals than plants. The proteins which compose the body of various animals differ. Likewise, those composing the body of different bacteria vary. The effects produced in animals suffering with various diseases are due in a measure to these foreign proteins entering the blood stream, where, according to Vaughan and Novy, they are broken into protein split products, some of which are toxic.

Another peculiarity of the bacteria is that they consist, to a large extent, of complex nucleoproteins and phosphoproteins. On hydrolysis, these yield the amino-acids, the purine bases, and phosphoric acid. The tubercle bacilli contain a typical nucleic acid of the animal type found in thymus, pancreas, sperm, and spleen. Whereas, the timothy-hay bacillus *Mycobacterium phlei* yields a plant nucleic acid similar to that found in yeast and wheat. Hence, from a chemical standpoint some bacteria have the characteristics of animals, others of plants.

Lipids.—Bacteria contain numerous organic compounds which are insoluble in water, but soluble in alcohol, ether, and chloroform, and which are greasy to feel. These are known as lipids.

True fats have been recognized in many species by special staining methods. By the use of fat solvents, lecithin, cholesterol, simple fats and specific bacterial fats have been isolated. The quantity and kind present depends upon the types of bacteria. Approximately 40 per cent of the dry weight of the tubercle bacilli consists of lipids. The amount varies with the strain, the bovine type being richer than the human type. It also varies with the media on which the organisms are grown. The fatlike substance (which the tubercle bacilli carry) prob-

ably acts as an armor, protecting the organism against adverse conditions, for these organisms are more resistant than most pathogens. Their peculiar staining properties are due to their high lipid content. Tubercle bacilli are very hard to stain, but when once stained they can be washed in dilute acids without removing the stain. They are, therefore, known as acid-fast bacteria.

Carbohydrates.—The most widely distributed and best known of the carbohydrates occurring in plants is starch, but the presence of true starch apparently has not been satisfactorily demonstrated in bacteria. Granules which are colored blue by iodine and are digested by amylase occur in certain bacteria. To these granules, Beijerinck has given the name *granulose*. These appear or disappear depending upon the age of the culture and nutrients present thus indicating that they are reserve food material.

Glycogen which bears the same relation to the animal as starch does to the plant occurs to only a limited extent in bacteria but may compose from 25 to even 40 per cent of the dry substance in yeast. Probably cellulose occasionally occurs in the body of the bacterial cell but this is the exception and not the rule. Probably in some cases the cell wall of the bacteria are composed of chitin, which on being digested with acids, yields from 80 to 90 per cent of a nitrogenous carbohydrate *glucosamine*. This is interesting in as much as cellulose is a typical vegetable, while chitin is equally typically animal in origin. The latter is found in the shells of lobsters and crabs and the wings and coverings of flies and beetles.

Ash.—The quantity of ash in the bacterial cell varies from 2 to 30 per cent, depending upon variety, age, cultural media and the conditions of growth. The ash of bacteria is usually considerably higher than in other plants. The same elements occur in both, but the bacteria are peculiar in the large quantity of phosphorus which they contain. At times over one fifth of the total ash is phosphorus, the remaining four fifths being composed mainly of potassium, sodium, calcium, magnesium, iron, sulphur and chlorine.

Composition of Different Parts of the Bacterial Cell.—Having studied the composition of the complete bacterial cell, let us now turn to an examination of the individual parts. Qualitatively this is done by microchemical means. For example, many of the proteins take up the basic aniline dyes, starch gives a blue

color and glycogen a beautiful reddish brown with iodine potassium iodide, whereas most fats give a brown or black coloration with osmic acid. The outer coating of the cell, the capsule, is a sticky gumlike mass which often causes the bacteria to adhere together. This consists principally of a protein mucin, similar to that found in saliva, or the slimy mass covering the body of snails. Sometimes as in the case of iron bacteria, they are surrounded by a sheath in which are large quantities of iron. Occasionally, such bacteria are found in water. Water containing these organisms when first exposed to air appears normal, but on standing the iron in the sheath is oxidized and the water becomes yellow. These organisms may form in large masses and clog the water mains; but from a sanitary viewpoint they are without significance. The cell wall varies, in a few bacteria it gives reactions characteristic of cellulose, hemicellulose, pectins, and other compounds related to the carbohydrates. In other bacteria the cell wall contains nitrogen and is composed of a chitin-like substance.

The inner part of the bacterial cell is composed largely of bacterial proteins. These vary widely with different species; the nucleoproteins and phosphoproteins probably predominate. Distributed through the cell are protein granules, carbohydrate granules, sulphur granules, fat globules, and crystals and globules of salts such as calcium oxalate, the quantity and quality varying with the variety of bacteria, age and conditions of growth.

Intracellular Granules.—In many bacteria, yeasts and molds intracellular granules occur which have been described under various names: "Metachromatic bodies," "Babes-Ernst granules," "volutin granules," "polar granules," and "Neisser's bodies." They possess a strong affinity for nuclear stains and assume a reddish-violet color when treated with methylene blue and certain other stains. They often disappear during active growth and reappear when growth ceases. The number and arrangement varies in different species and may appear at the poles; hence, the name "polar bodies." They have been credited with almost every conceivable function. The early discoverer believed them to be a part of the nuclear material of the cell. Other observers considered them to be early stages in spore formation, and still again they have been considered structures comparable to the centrosomes of other unicellular organisms. At one time they were believed to indicate a high degree of

virulence on the part of the bacterium. Even today the true nature of the bodies is not definitely known. They appear to be reserve nitrogenous food material, either nucleic acid or nearly related compounds. They are so characteristic of some micro-organisms (*i. e.*, the diphtheria bacilli) that they are used in microscopical differentiation.

Protoplasm.—We have seen that it is a peculiarity of living matter to be always found in the organized form; that is, the body of the living organism is composed of organs, the organs of tissue, the tissue of cells. As we have seen, even the cell is composed of definite organized parts. This organized mass of living material composing the cell is spoken of as protoplasm. It is not, as was formerly believed, a definite chemical entity, but is composed of the various substances which enter into the vital construction of the cell. It in turn is organized. Just as the "Milky Way" of the heavens dissolves into individual stars when viewed through the telescope, so in a similar manner the cell protoplasm (highly magnified) shows organization within organization. Yes, it may be that it is not only organized but peculiar to the specific cell. All recognize that there are no two men exactly alike. The cattleman tells us that he has no two cattle alike; the sheepherder that there are no two sheep alike; the botanist that no two leaves or blades of grass are alike; and now the biologist tells us that the protoplasm composing our tissues is different from that composing the tissues of other individuals. Our individuality goes back to each individual cell. True, proteins are composed of the same amino-acids and protoplasm of the same proteins, fats, and carbohydrates, but these in turn are arranged in different orders. Calculating the theoretical number of permutations and combinations we find that there are countless billions of different protoplasms possible. These, while the stream of life is coursing through the living cell, are held in certain labile positions, but when death comes they swing back to the stable. Hence, it may be that in this sense individuality exists even among the bacteria.

CHAPTER XII

CHEMICAL ACTIVITIES OF BACTERIA

BACTERIA are master chemists which smoothly and orderly analyze and synthesize in a compact bit of protoplasm a great variety of compounds; while so doing, they build and repair their own bodies. They work by means of enzymes and produce an interesting variety of chemical compounds, some of which are far more complex than any produced by man.

Enzymes.—Enzymes are complex organic compounds which accelerate chemical reactions without themselves being used up in the process. They are secreted, probably in the inactive form, by living cells. The inactive form, zymogen, is rendered active by various substances, often acids. They act on various substances referred to as the substrate and yield either more or less complex compounds, depending upon the enzymes and conditions under which they work. They are the tools with which bacteria do their work. With them micro-organisms break down the carbohydrates, fats, and proteins. They are also used in coagulating milk and probably in most other changes which microbes bring about.

Specific enzymes are used for specific purposes. Those which decompose fats are different from the ones which decompose carbohydrates, and the ones which act on milk sugar have no appetite for cane sugar. Bacteria use enzymes in the digestion and metabolism of their food; consequently, if they have one enzyme they can digest this food; if another, that. Hence, we may say that the enzyme determines the appetite of the microbe. What peculiar appetites microbes sometimes have! Some digest horn, others wood, and still others rock. In many cases, the enzymes diffuse out of the cell and bring about their characteristic reaction in the surrounding media. The liquefaction of gelatin is due to such an *extracellular enzyme.* In other cases, the reaction occurs within the cell due to *intracellular enzymes.* Yeasts produce alcohol with an *intracellular enzyme,* zymase.

The modern system for naming enzymes is to add the termination "ase" to the root word referring to the more complex substance on which the enzymes act. Consequently, the enzymes

which act on proteins are referred to as proteinases. The terms, "sucrase," "maltase," "lipase," "glucase," and "glycogenase" are similar self-explanatory names. Many of the enzymes, early in the history of physiology, received special names, as "ptyalin," "pepsin," and "trypsin." Each of these names was chosen by an early writer in accord with what seemed to him a suitable characterization. Even though these names persist in the literature of today, they can be named according to the modern system. Ptyalin would then become salivary amylase, pepsin gastric proteinase, and trypsin pancreatic proteinase.

A peculiar property of the enzyme is its great activity. A very minute quantity can bring about tremendous changes. Theoretically, a thimbleful of the enzyme which digests proteins can break down thousands of pounds of protein, provided it were given the time and no accident happened. If we double the quantity of enzyme we roughly reduce the time by one half. The reason for this is that each particle of enzyme repeats the same kind of work over and over. It is somewhat similar to a group of men carrying bricks from the ground to the top of a building. Give one man sufficient time and he would be able to transfer the whole pile to the top eventually, but in case many men help in this task, the time required to carry the bricks to the top would decrease as the number of men increased. Occasionally, however, the time used in the work is not directly proportional to the number of men starting. A brick may slip, strike one of the men on the head, and he is through carrying bricks—at least for the time being. A similar phenomenon occurs with the enzymes. Some of them may get entangled in some side issues, and their work is at an end. Moreover, the speed with which an enzyme works depends upon the temperature. Increasing the temperature up to a certain point results in an increase in its activities. Above a certain degree, however, the activity decreases, and if the temperature becomes too high the enzyme is destroyed. Like animals, enzymes are destroyed by poisons. During the life of the bacteria these enzymes are working in harmony, each performing its allotted task —some building up this part of their home, the cell; others, preparing the fuel; and still others, doing away with waste products. Just the minute life ceases, each enzyme commences to work independent of all others, and in time their home (the cell) is torn to pieces. This is likened unto a dance. As long as the music continues, all are in step and there is a certain

beautiful rhythm in all. When the music ceases, however, the dancers lose step, bump into each other, and if they continue, all becomes confusion. The enzymes are the dancers in the living cell. Life is the master musician who furnishes the music. Hence, enzymes manifest themselves in the living and in a degree are subject to the limitations of life; but they are not life.

Pigments.—Color is to the eye what music is to the ear. Abstract it from plants and animals and they immediately become less interesting. Consequently we would expect to find colored bacteria and in this we are not disappointed as most micro-organisms possess some pigmentation. In the *chromogenic* bacteria the colors may even rival those of the rainbow.

There is a long mysterious, romantic history connected with the appearance of "bleeding" bread, "blood drops" on sacred wafers and an outbreak of "bleeding" polenta (corn meal mush) which disappeared only when man grew in the laboratory a tiny organism, *Serratia marcescens,* sometimes known as *Erythrobacillus prodigiosus.* Sometimes, today, bread becomes infested with this organism, and dough which has been set aside overnight not infrequently has been found in the morning to fairly rival the colors of the autumn sunset.

At times on standing, milk develops a very uncanny blue color, which spreads through the dairy like an epidemic. The little culprit which plays this trick is the *Pseudomonas cyanogenes.*

In the soil are some nitrogen-fixing bacteria which produce a golden-yellow pigment, while others form a chocolate-brown. Some, in water, produce green and various other colored pigments. Occasionally pigment production occurs in organisms which do not normally possess this power. For example, the diphtheria bacillus rarely produces an orange-yellow pigment and the tubercle bacillus a yellow or reddish pigment.

A few bacteria contain within their bodies a red-purple pigment, *bacteriopurpurin,* which enables them to assimilate carbon dioxide by the aid of the absorbed sunlight. Many attempts have been made to explain the function of the other pigments; but so far none seems to be wholly satisfactory. The color seems to be of no material advantage to the bacteria, for colorless strains may be cultivated which possess all of the properties of the original strain, with the exception of pigment production. There is no evidence that they protect the organism against light, nor is there anything that would lead one to believe that

they are similar to the hemoglobin of the blood, or the chlorophyll of the green plants. The best evidence, therefore, points to the conclusion that they are mere by-products which have no particular value to the organisms.

Freshly isolated organisms often produce pigments in abundance and lose this property after cultivation on artificial media. Certain media such as potatoes enhance color production. Pigments are usually produced at low temperatures under aerobic conditions; rarely are they produced under anaerobic conditions.

Toxins.—Bacterial poisons, toxins, are of two classes: Exotoxins and endotoxins. Exotoxins are produced by bacteria and diffuse into the surrounding medium and can be separated from the living cells by filtration. Typical examples of bacteria which produce exotoxins are: The diphtheria, tetanus, and botulinus bacilli. The production of the diphtheria toxin is dependent, in a degree, upon the food of the organism. This bacterium, grown in beef tea, produces a highly potent toxin, whereas the same organism grown in the presence of glucose produces lactic acid; hence, as pointed out by Kendall, "The story of Dr. Jekyll and Mr. Hyde, that strange and imaginary conception of a dual human personality, has its actual realization (and far more striking and realistic) in the simple experiment upon energy of the diphtheria bacillus. In plain broth the microbe produces a potent toxin which confers on the bacillus its formidableness in producing disease. The simple addition of energy for the organism so changes the nature of its growth that it is not only no longer toxic, it is potentially possessed of food value."

Exotoxins have characteristics which differentiate them from other poisons:

1. Toxins are specific, that is, the diphtheria bacterium produces a poison which, if placed in the body of a susceptible animal, will give rise to diphtheria. We can grow the diphtheria bacteria in beef tea and filter off the bacteria; yet this beef tea if given to a susceptible animal will cause the disease diphtheria. Diphtheria organisms alone produce diphtheria toxin; lockjaw organisms produce lockjaw, etc. Hence, they are specific.

2. Toxins are extremely violent poisons, far surpassing any other known poisons in this respect.

	mg.
Smallest quantity of atropine required to kill a man	130
Smallest quantity of strychnine required to kill a man	30–40
Smallest quantity of cobra venom required to kill a man	4.4
Smallest quantity of tetanus toxin required to kill a man	0.23

The tetanus toxin is produced by the bacillus which causes lockjaw. The poison has never been obtained pure, yet the crude natural product is highly poisonous. What a minute quantity of the pure compound would be required to kill a man! Other toxins are even more potent. The minimum lethal dose of a potent diphtheria toxin for a guinea-pig is about 0.002 cc., of a potent tetanus toxin about 0.0005 cc., and of a potent botulinum toxin about 0.0001 cc. It would appear that only a few molecules of the botulinum toxin, a poison sometimes produced in food, is required to kill small animals.

3. Bacterial toxins, if used in extremely small but increasing doses, will produce immunity. When a very minute trace of the toxin enters the body of an animal, the cell begins to manufacture an emergency material which robs the toxin of its power. This is known as antitoxin, and the presence of it in the blood of the individual in sufficient quantities means that he will not take lockjaw, diphtheria, or whatever the specific disease. We then say he is immune. The resistance may become so great that it makes no difference how many of these poison darts are shot at the individual by the microbe; they fall without piercing his armor of immunity. However, it is different with strychnine. To this, the individual never becomes resistant. Man may become able to tolerate more nicotine, but give even the habitual tobacco user a large dose of nicotine and death will result.

4. Toxins have an incubation period, whereas most ordinary poisons have not. If an animal receives strychnine, it soon becomes ill and quickly recovers or else dies; whereas if an animal is given a bacterial toxin there may be a delay of days or even weeks before the symptoms appear. The time between the entrance of a toxin into the body and the appearance of the symptoms is the incubation period. It varies with different toxins and also with the quantity administered. In the case of *Staphylococcus aureus* toxin, the incubation period may disappear. While maximal doses of diphtheria toxin fail to kill a guinea-pig in less than ten hours, yet 0.05 cc. of staphylococci toxin will kill a rabbit in less than three minutes.

5. Toxins are easily destroyed by drying, sunlight, heat, and certain chemicals. Thus, any material containing bacterial toxin, if heated to 100° C. for fifteen minutes, will be free from it. The toxin produced by the *Streptococcus scarlatinae* is somewhat more resistant. Most toxins are destroyed by the gastric juice, the botulinum toxin being an exception. A toxin may be

heated or treated with a chemical and thus lose its power of poisoning an animal, and still retains its power of calling out "antitoxin," thus rendering the animal resistant to disease. This practice is used extensively in medicine to protect us against disease.

Endotoxins are produced within the bacterial cell but do not diffuse into the medium in which the bacteria are growing. The usual method of preparing a solution or suspension of an endotoxin is to disintegrate the bacterial cells by prolonged grinding or alternate freezing and thawing, or by permitting the cells to autolyze in a fluid medium. It is assumed that when the bacteria enter the body of a susceptible animal they are broken down with the liberation or production of endotoxins. They do not give rise to as clear-cut symptoms; they are more resistant to heat; and they are not nearly so toxic as are the endotoxins. There are exotoxins which are one million times as potent as are the endotoxins. They do not call out as regularly in the body of the recipient antitoxic substance, and with some endotoxins it has been impossible to prepare anti-endotoxin serum.

Putrefaction.—Proteins are highly complex compounds composed of carbon, hydrogen, oxygen, nitrogen, and sulphur. For the most part they belong to that group of compounds referred to as colloids. On digestion they are broken into specific products, amino-acids, some eighteen or twenty of which are known. Bacteria break down proteins, and thus produce from them these amino-acids. However, they go a step farther and disrupt the amino-acids with the production of ill-smelling compounds. Hence, the idea became prevalent that putrefaction is literally a rotting or offensive decay of proteins. Pasteur first considered it essentially an anaerobic process brought about by various bacteria. The term is also used in a more general sense to signify the decomposition of protein material due to the action of micro-organisms. The end-products of putrefaction are ammonia, nitrates, carbon dioxide, hydrogen sulphide, and the like—all simple, stable nonpoisonous compounds in the ordinary concentration. However, the intermediate products resulting from the partial decomposition of proteins may be toxic. The quantity and nature of these products depend upon the specific bacteria and the media in which they are growing. In this group are the so-called "ptomaines." They have been defined by Vaughan as organic chemical compounds, basic in character, and formed by the action of bacteria on nitrogenous matter. Most of them

are nonpoisonous. Two thirds of them contain only carbon, hydrogen, and nitrogen. The others also contain oxygen. These latter are the more poisonous. Some members of this group are: Sepsin, cadaverine, putrescine, choline, muscarine, and neurin. The last two, which are quite similar in action, are very poisonous, and have been obtained from poisonous mushrooms. It is possible that they also play a part in cattle poisoning which sometimes results from the feeding of fermented beet by-products.

Light.—Most individuals have been mystified and delighted, if not frightened by the uncanny, beautiful light which sometimes covers a stump in the forest. At times, especially along the seashore, it seems to be emitted as a mysterious yet beautiful phosphorescence from the sand. One writer tells of a cluster of sausages brought to his laboratory. It was hung up in a dark cellar and when the maid went for it in the morning there "hung in the place of the sausages a fiery effigy which seemed to her more like the quondam spirits of their mysterious ingredients than the unctuous homely friend of the homeless boarder."

The explanation of these apparent mysteries is that certain bacteria live on decaying wood, leaves, salt water, meat, and fish, and emit a light. Some 20 species have been described which possess the power of emitting light, some of which will retain their luminescent power for hours after death.

The production of light depends on the temperature and food supply. The phenomenon has been seen at temperatures as low as —20° C. and as high as 45° C. The luminous bacteria seem to like salt and to require oxygen, for when the latter is exhausted in their media, the luminosity ceases. If a bubble of air is made to pass up the side of a tube containing a culture of luminous bacteria, which has just ceased to emit light for lack of oxygen, a wave of light may be seen to pass along the tube. However, the actual quantity of free oxygen required is very small. In some cases, one part of oxygen in about 143,000 parts of hydrogen by volume was found sufficient. This corresponds to one part oxygen by weight dissolved in 3,700,000,000 cc. of sea water.

Beijerinck has suggested that photogenic bacteria may be used to test the soundness of porcelain filters, the effect of light of different intensities on photosynthesis, and the presence of certain carbohydrates. Some micro-organisms will produce light when grown on one carbohydrate but not when grown on another.

Certain fishermen employ, as bait, luminous fragments of dead fish, made so by bacteria.

Strains of organisms have been produced by selection so luminous that their light could be seen in full daylight. It has been possible to obtain sufficient light from some of them to permit the reading of time from a watch or moderately large print in dark rooms. Lode calculated that the organisms with which he was working if used to coat the entire dome of St. Peters at Rome would give off little more light than an ordinary tallow candle, but other workers have obtained cultures ten times as photogenic.

Bunge and Neill exposed various micro-organisms to ultraviolet light and found that the nonfluorescent were killed more readily than the fluorescent bacteria. They believed that the fluorescent bacteria protected themselves against the coagulating effect of ultraviolet rays by converting the shorter wavelengths into longer waves, thus disposing of the energy of the absorbed short waves which would otherwise be spent in coagulating the bacterial proteins. The nonfluorescent bacteria are unable to do this and hence succumb.

Heat.—Probably all bacteria liberate heat; there are a number which liberate it in sufficient quantity to change the temperature of the medium in which they grow. This is observed in the heating of fermenting silage, manure, and hay. At times, the temperature is raised to the kindling point, with the result that spontaneous combustion may occur in hay and grain stacks. Bacteria generate considerable of the heat, but other chemical processes are also active. This is made use of in the building of cold frames. A heavy dressing of fresh horse manure is placed under the top soil. This decomposes and furnishes sufficient heat to force the plants.

CHAPTER XIII

FOOD REQUIREMENTS OF BACTERIA

All living organisms require food. Some forms require it only for growth and repair, whereas others need it also as a source of energy. The energy of sunlight is locked up in chemical compounds by green plants. These, when used as food, are decomposed and built into specific protoplasm, or the energy is liberated for use in life activity. Bacteria are chlorophyll-free plants; hence, they require food for growth, repair, and energy.

Food.—The term, food, in its broadest sense, may be considered as including everything taken into the body which is essential to the life of the body and its activity. In this sense, carbon dioxide, water, and the mineral elements are often spoken of as the food of plants. They are useful only to the green plant and to these only in the presence of light. To chlorophyll-free plants (or to plants in the dark, and to all animals) they are not food. For this reason, it is stated that green plants build their own food from the simple inorganic constituents—water, carbon dioxide, and the minerals of the soil. Thus are produced storehouses of potential energy which are used by other organisms not so favorably constituted.

Bacteria, with the exception of a few possessing a purple coloring matter *bacteriopurpurin,* are unable to use the kinetic energy of the sunlight in the production of their food, but some of them can utilize that of inorganic compounds, and in the presence of the required elements build their own food. However, the majority of bacteria obtain their food, ready formed, from other plants or animals; hence, from the viewpoint of bacteriology, we may consider food as any substance which bacteria can utilize in obtaining either building material or energy for cell activity. It may be that in the future this definition will be enlarged to include substances which are taken into the body and govern cell activity, as is the case with the animal cells.

Although the forms of food required by different bacteria vary greatly, they must all contain ten of the known elements. These, all of them very common, are: Carbon (C), hydrogen (H),

oxygen (O), nitrogen (N), calcium (Ca), potassium (K), magnesium (Mg), sulphur (S), phosphorus (P), and iron (Fe). Using the key for remembering, as suggested by that great teacher, Cyril G. Hopkins, we have: C. Hopk'ns Ca, Fe, Mg—"C. Hopk'ns Cafe—Mighty good."

Food may be divided into three great classes: Organic, ash, and water. The organic compounds comprise the four great groups: Carbohydrates, fats, proteins, and vitamins. The first two are composed of carbon, hydrogen, and oxygen; whereas, the proteins contain in addition, nitrogen, sulphur, and sometimes other elements.

Quantity of Food Required.—Bacteria are like animals in that the quantity of food needed and that actually used in the presence of an abundant supply is widely different. Some bacteria will actually multiply in distilled water. In such water, they may multiply until there are billions in a gallon, obtaining the food and energy for their life processes from the minute traces of impurities present. Other bacteria will grow on the surface of granite, obtaining their required food from the small quantity of foreign matter brought to them in the rain water.

On the other hand, the maximum quantity of food used by bacteria may be enormous. They quickly decompose the bodies of dead plants and animals. Tons of material are carried into the septic tanks of large cities, all of which are rapidly used by bacteria.

The food required by each bacterial cell is not great, for it would take 1,600,000,000 colon bacilli to weigh approximately 1 mg. The waste products and repair material would make the cellular requirements slightly greater than this, but the figures are sufficiently accurate to indicate that the quantity needed for building material for one cell is exceedingly small. Their great need for building material is brought about by the rapidity of their multiplication. Colon bacilli placed in favorable environments may divide into two daughter cells every fifteen minutes. The theoretical twenty-four-hour progeny of a single colon bacillus therefore would be 2^{96}, a truly enormous number—a number which would have a very appreciable mass. After the first few hours natural barriers (such as the lack of food, accumulation of waste products, and mutual antagonism) retard the multiplication of bacteria. Nevertheless, the rapid multiplication of bacteria is one of the factors which make their food requirements so great.

As pointed out by Kendall, another noteworthy characteristic of the bacterial cell is its disproportionately large ratio of surface area as compared to weight or volume. Thus, the typhoid bacillus which is a microbe of average size, has a volume of approximately 0.000,000,000,5 c.mm. and a weight of about 0.000,000,000,5 mg. but a surface of 0.000,003,5 sq. mm. In no other living organisms (except possibly the filtrable viruses) is this ratio of surface to volume equaled. The energy requirements of living things in general are determined to a great extent by the ratio of their surface to their volume. Hence, the energy requirements of bacteria are great. Moreover, many only partly digest their food, leaving most of the energy in the by-products. For instance, micro-organisms, which completely oxidize sugar to carbon dioxide and water, will obtain thirty times as much energy from a given quantity of grape sugar as will others which change it only to alcohol.

How Bacteria Feed.—Animals take food into their stomachs and secrete digestive juices, which are mixed with it and render it soluble and assimilable. It is then taken up by the blood, carried to the different parts of the body, and there used to build new tissues, to repair old worn out ones, or to furnish energy to the animal. Bacteria have no stomachs; consequently, they pour their digestive juices directly into the medium in which they are living. After digestion, the food is absorbed. This very fact makes the bacteria of economic importance, as many of the digestion products are of value to man; moreover, some of the food of plants are digestion products of the bacteria.

Kinds of Foods Required.—Bacteria feed upon a great variety of substances. The sulphur, iron, and nitrifying bacteria are able to live upon mineral nutrients. The sulphur organisms obtain their energy through the oxidizing of sulphur dioxide to sulphuric acid. The nitrifiers oxidize ammonia to nitrites and then to nitrates. Both classes obtain their building material from simple inorganic compounds; consequently, they can live without plant or animal tissue, in the absence of sunlight. It is this function that leads us to believe that they probably were the first inhabitants of this globe. The majority of bacteria cannot live upon inorganic compounds, but like animals require more complex foods. The ordinary decay bacteria thrive on any plant and animal tissue. Woody fiber, bone, horn, and proteins of all kinds are rather rapidly consumed by them. Other organisms, as for instance those causing tuberculosis, are more

particular in their food requirements, growing best on blood and tissue extracts. Others, as for instance the leprosy organism, appear to grow only in living tissue.

Those bacteria which require neither organic carbon nor organic nitrogen, but live upon minerals, as do the higher plants are often referred to as *autotrophic* bacteria; those which feed upon dead plants and animals are called *heterotrophic* or *saprophytes;* and those which feed upon living plants and animals are referred to as *parasites.* The first two classes contain the beneficial soil organisms, whereas the parasites are the disease producers. This latter group is often spoken of as the pathogens. A micro-organism may live at one time as a parasite, and at another as a saprophyte. Some few bacteria appear to live only as parasites. In time, we may learn how to grow all so-called "obligate parasites" on artificial media.

Water.—Man can live for days without food, but only for a few hours without water. The same is true of bacteria. Remove all the water from the medium in which bacteria are growing and they soon die or pass into the spore stage. The pneumococcus, the cholera spirillum, and the Pfeiffer bacillus die in a few hours on drying; whereas the typhoid, diphtheria, and tuberculosis organisms may survive for days. The tetanus, anthrax, and many soil organisms resist drying for months or even years. Of course, even in the case of these resistant organisms the water is never completely removed; otherwise, they would quickly die.

Water plays a number of parts in bacterial life: (1) It dissolves the food so it can be taken up by the bacteria. When the food is not already soluble the microbe throws off an enzyme which brings the food into solution so it may be taken up by the cell. (2) Water carries the waste products from the cell. (3) It enters into nearly all the changes going on within the cell. When starch is being digested, water enters and the starch breaks into sugar. Fats break into glycerin and an acid through the taking up of water. In the digestion of proteins, every step is accompanied by the taking up of water. When bacteria are building up in place of breaking down, water is the pivot around which all reactions turn. When food burns within the body or within the stove, water is continually being evolved. (4) Water is the agent that speeds up all reactions. If a match becomes wet, it will not ignite; nor will it if the air, match and surface on which it is rubbed are absolutely dry. It is the same with

the changes going on within the cell. Water must be present or the cell reactions will not proceed normally. (5) Water enters largely into the composition of the cell, as bacteria consist of from 75 to 98 per cent water. (6) Water gives to the cell its shape. If we place a cell in a strong salt solution, water is drawn from it. If we place it in distilled water, it bursts. The force which causes the bursting is called osmotic pressure. The extent of this pressure varies with different cells. It averages about the same as that of a 0.9 per cent salt solution. This is about 7.1 atmospheres. In such a solution, the tissues neither gain nor lose weight.

Carbon Requirements.—All living protoplasm require carbon. But, how varied are the forms in which it is taken! Green plants take it up as carbon dioxide; man, as carbohydrates, fats, and proteins. Probably all forms of carbon, except the diamond, are utilized by some bacteria. Some soil organisms can utilize the carbon of carbon dioxide; others, that of formaldehyde; while still others can utilize the carbon of carbolic acid. Both soil and manure contain bacteria which use the carbon of urea; from some of it they construct their bodies, but most of it is changed into ammonia which is used by other microbes. The woody fiber of plants yields its carbon to bacteria, and there are writers who claim that coal is produced by the action of bacteria upon plants. Renalt, for example, describes fossil bacteria which he found in coal, and today we know microbes which get their required carbon from coal, and also bacteria that produce the coal-like material occurring in soil. Most bacteria, however, prefer their carbon in the form of carbohydrates. Many pathogens growing in the presence of them produce nontoxic acids, but if grown in the absence of carbohydrates produce very potent toxins. Bacteria are very fastidious in their carbohydrate requirements. Some bacteria will pick all of one sugar from a medium and leave untouched a nearly related compound. This characteristic is often made use of in identifying or separating nearly related compounds. Such reactions are far more delicate than those made by the most refined chemical reagents. For instance, available information indicates that as small an amount as 0.0025, or possibly 0.001 per cent, of glucose in an appropriate cultural medium may be detected by the use of suitable bacteria. This method can also be used to identify bacteria by seeding the unknown

organisms into the various sugars and noting the one which it digests.

Proteins are split by some bacteria, and the carbon of the resulting amino-acids utilized for energy or building material;

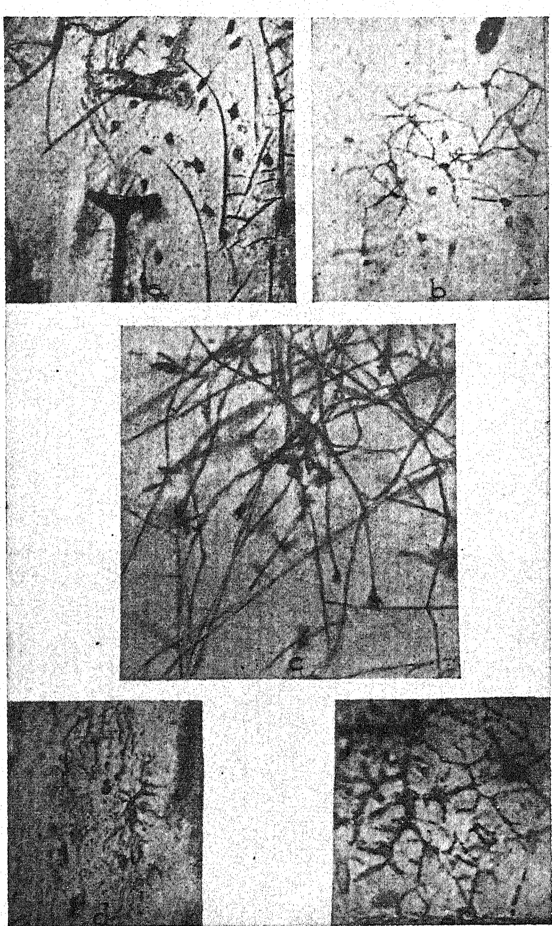


Fig. 48.—Carboniferous bacteria and fungi as figured by Renalt. (*After Moodie.*)

however, many bacteria cannot split the protein molecule and only utilize the carbon of proteins after they have been broken into amino-acids by animals or other bacteria.

The carbon in the fat molecule is more resistant to the attack of bacteria. For this reason, it often gives considerable trouble to the plumber. Fatty materials are thrown into the sink; they find their way into the sewer; bacteria dissolve the accompanying substances and the fat is liberated. This collects around some object, as for instance, a match. As this is rolled along by the water it gathers more fat, until at times it becomes large enough to clog a pipe. Proteins yield their carbon readily and often in the breaking down of them, as for example in the egg, the sulphur is changed to the ill-smelling gas, hydrogen sulphide. A few of the pathogens require their carbon as the complex body tissues and fluids, and a very few at the present time seem to require that this tissue or fluid be in the body of a living animal.

Some of the carbon is used by the microbe in building cell protoplasm. However, most of it is used as a source of energy. Inasmuch as the energy requirements of micro-organisms are relatively great, carbon is required by them in larger quantities than is any other element.

Nitrogen.—Nitrogen in the free form is one of the inert elements. However, when once cajoled into joining hands with carbon and oxygen, we obtain wonderful compounds. The most beautiful dyes contain nitrogen. It is tucked away in the most powerful explosives, ready on the least provocation to become excited and blow everything to pieces. The majority of delicate perfumes and powerful poisons contain it. But most wonderful of all, it is the basis of living protoplasm! All micro-organisms require it in one form or another. The nitrogen-gathering organisms of the soil can cause the inert atmospheric nitrogen to join hands with carbon, hydrogen, and oxygen and thus produce their mysterious bodies. There are bacteria in manure which transform urea into ammonia. Others oxidize the ammonia to nitrites, and still others, in obtaining their energy transform the nitrites into nitrates. Many bacteria can use the nitrogen in egg, milk, flour, and all kinds of plant and animal tissue; others require more complex nitrogen compounds, as for instance such as are found in living plant and animal tissue. Sometimes the protein compounds are pulled to pieces with the formation of ptomaines; at other times, they are built into those most powerful of all poisons, toxins. Much of the nitrogen, however, is used in building that wonderful little engine, the body of the microbe. Micro-organisms break down the complex

proteins into simple building blocks before they are built over into their bodies just as man would reduce the wall of an old church before building it over into a new schoolhouse. Soil bacteriology deals with the soil organisms and to a great extent their relationship to the nitrogen supply. Many bacteria are busy changing the nitrogen compounds into forms which are acceptable to the higher plants.

Oxygen.—Oxygen is essential to all forms of life. Some bacteria require it free, others can use it only when combined. To those which can live only in free oxygen, Pasteur gave the name, *aerobes* and to those which live only in the absence of free oxygen, *anaerobes*. Some can become adapted to the use of combined oxygen. These are known as *facultative anaerobes*. Free oxygen is a poison to absolute anaerobes. True aerobes are great lovers of oxygen; and if shut up within a little cell containing a few drops of water in which is enclosed a bubble of air, on examination under the microscope it will be seen that the motile ones will make their way from all parts of this tiny ocean and cluster around the bubble of air.

Hydrogen.—Hydrogen is required by all micro-organisms. There are some which can use hydrogen gas, but most of them obtain it in combinations such as water, or the various organic compounds.

Ash Requirements.—Animals fed on a diet of pure water, ash-free proteins, carbohydrates, and fats die sooner than if given only water. Likewise, bacteria require mineral nutrients. They use them for various purposes: (1) For maintaining the osmotic pressure of the cell. These give to it its plump contour and assist in the taking up of foods. (2) For the neutralizing of acids. The nitrifying bacteria and many other micro-organisms produce acids, which, if permitted to accumulate, would soon cause the death of the cell. (3) Some of the minerals are built into the body of the microbe and give to it its peculiar chemical and physical properties. Some bacteria use sulphur for energy. These are comparatively large organisms and have when taken directly from sulphur springs yellow granules of sulphur within their bodies. On fasting the bacteria, these granules soon disappear, and sulphur dioxide, hydrogen sulphide, or sulphuric acid appear in their place, depending on the specific micro-organism in question.

Vitamins.—Vitamins are essential to the well-being of all animals. They are produced by green plants, and there is evidence

today that bacteria can also synthesize them. Although they may not be essential to bacterial growth, there are, nevertheless certain accessory food substances which exert a favorable influence on bacterial development. Davis and Ferry describe a remarkable stimulating influence on the growth and toxin production of the diphtheria bacilli by the addition of small quantities of vitamin-containing substances to the medium in which the bacteria were growing. Several other workers have likewise noted a somewhat similar stimulation in the growth and development of the azotobacter and streptococci. The authors have showed that *Azotobacter chroococcum* can synthesize the precursor of vitamin D.

Mutual Relationship Between Plants.—There are some species of micro-organisms which live in close association with other plants and in this manner get their food. The legume bacteria live in the small nodules of the alfalfa plant, drawing from it the food needed, and giving the alfalfa nitrogen compounds in return. Such a friendly association plays a prominent part in soil bacteriology, for probably there are many micro-organisms which thus associate with one another. Hence when two or more organisms are living together and receiving mutual benefit it is referred to as *symbiosis*.

Sometimes, one microbe changes the food so that another can readily use it. There are bacteria in the soil which break down plant tissues into acids which are used by the nitrogen-fixing bacteria. Yeasts act on sugar and change it to alcohol. This can be used by bacteria in producing vinegar. Such an association is known as *commensalism* or in some instances as *metabiosis*.

There are other species of bacteria which are mortal enemies and cannot live together, one species killing out the other almost as soon as they come in contact with each other. The details of this miniature warfare are not well understood, nor do we, in all cases, know the weapon with which it is carried on. It is probable that some poison is secreted which is without effect upon its producer, but deadly to its antagonist. This is well illustrated by the following example. Meat if left by itself soon spoils, but if dropped into buttermilk will keep some time. The lactic acid formed by the milk organisms is a deadly poison to the putrefying bacteria. This antagonistic action is called *antibiosis*.

Occasionally two bacteria growing together can form products which can be produced by neither growing alone. Some

micro-organisms growing in pairs on a carbohydrate produce a gas whereas either growing alone produces no gas. Kendall found under normal conditions *Escherichia coli* does not produce gas in milk but when grown in milk with a strong proteolytic microbe gas is produced in considerable quantities. Such a condition is known as *bacterial synergism.*

CHAPTER XIV

PRODUCTS OF BACTERIAL ACTIVITY

Bacteria are of economic importance for three reasons: (1) Many act as scavengers, clearing away the dead and useless bodies of plants and animals. (2) Some bacteria produce products which are valuable to man. A number of these substances are used directly. Some favorably influence man's food, while still others prepare food for the growing plants. (3) A few bacteria produce compounds which are poisonous to man and the lower animals.

Metabolism.—Living organisms are peculiar in that they can take materials very unlike their own bodies and build from them their specific tissues. They also can liberate the energy contained within organic compounds and use it in their body activities. This necessitates the changing of the substances. Sometimes it is a building of more complex from simpler compounds: *Anabolism,* or *anabolic* processes. More often it is a breaking up of complex compounds into simpler substances: *Catabolism,* or *catabolic* processes. The total transformations going on in the plant, or animal body are known as *metabolism,* a term which really means "transformation of matter."

The metabolism of bacteria has commanded the attention of scientists of recent years not only because of the economic importance of the resulting products, but also because it opens the way to a better understanding of the transformations going on in the human body. Bacteria use the same foods as man. From them they build complex compounds, and what is more important, they oxidize them. Man is able to obtain these oxidation products from the bacteria long before these products have reached the final stage of carbon dioxide and water. From a study of these fragments scientists are formulating theories as to the way in which the cell works in obtaining its energy. This has resulted in working theories which can be applied to the animal organism.

Chemical Products.—Jordan divides the products produced in bacterial life into four classes: (1) Secretions, (2) excretions, (3) disintegration products of bacterial activity, and (4) true

cell substances. The secretions, those substances which serve some useful purpose in the cell activity, may be retained within the cell or pass into the surrounding medium. Enzymes are typical examples of these products and have been considered in a previous chapter. The excretions, those substances which have been ejected as useless to the cell, are the ashes of cell metabolism. Nitrous, nitric, and carbonic acid are examples. The disintegration products of bacterial activity consist of the various organic acids, nitrogenous constituents, and ptomaines. The true-cell-substance group includes the protoplasm and the products which are transformed into protoplasm.

Fermentation.—The term "fermentation" has been variously used. It was originally applied to any type of bacterial activity in which carbohydrates or nitrogenous substances were broken into simpler products. It was used almost synonymously with the term "decomposition." Later, chiefly due to the work of Fischer the term "fermentation" came to imply *the biochemical decomposition of nitrogen-free compounds, chiefly carbohydrates, due to the actions of micro-organisms.* Sugars, starches, celluloses, and their derivatives are broken down in this process with the formation of alcohols, acids, and other substances, and finally carbon dioxide and water. There is usually an absence of ill-smelling and toxic substances which are prone to occur in putrefaction.

Alcohol.—Alcoholic fermentation is due principally to yeasts. Some molds and very few bacteria also produce it. When alcohol is formed by bacteria it is produced only in small quantities and always accompanied by several acids. In fact, as produced by all micro-organisms it is associated with other products: glycerin, fusel oil, succinic, acetic, and lactic acid. The quantity and kind of each product depend upon the material being fermented and the specific micro-organism bringing about the change.

The classic work of Pasteur on the so-called "diseases of wine," led to a definite knowledge of the processes involved in the making of alcoholic liquors. In the alcoholic fermentation of fruit juices, sugar is the substance decomposed. In bread dough and in beer-wort it is malt sugar (maltose derived from the flour of the germinating grain). The same is true of corn and potato mash (from which Bourbon and Irish whiskies are obtained). The variation in wines produced in different districts is due to the specific micro-organisms which find their way into the fer-

menting mixture. There are strains in some districts superior to those found in others just as superior strains of flowers and plants occur in various localities. However, the modern manufacturer does not depend upon chance seeding, but grows within his own laboratory the superior strains which are used to inoculate the substances to be fermented. Various substances may be used in the manufacture of alcohol. In this country the chief substance is sugar-cane molasses; in Germany, potatoes; in France, sugar beets; and in many other countries, sorghum is used.

The sugar-cane molasses is diluted with water and a little acid is added in order to give the yeast better working conditions. A pure culture of yeast is added and fermentation permitted to continue for about three days. The carbon dioxide given off is collected, purified, and stored in cylinders to be sold for use at soda fountains. The resulting mash contains from 6 to 8 per cent alcohol, which is pumped into a still and distilled. This crude distillate contains many impurities—aldehydes, esters, and higher alcohols. These are separated by rectification. By this means, alcohol of a very high state of purity is obtained.

In the first stage of vinegar production, alcohol is produced by yeast, which is later oxidized to acetic acid by bacteria.

The various changes which occur in dough that is made from flour, and before it is baked, are known as panary fermentation. The product formed consists essentially of gas, usually as a result of alcoholic fermentation brought about by yeast. However, there are other products, especially acids, the nature of which depends upon the leaven used in making the dough. During the leavening process, the gas is produced which causes the porous light condition of the resulting bread. Products which contribute to the flavor are also formed. The baking destroys most of the micro-organisms and drives off much of the alcohol, but the lactic acid, which has been produced, remains.

In parts of Europe and Asia preparations containing alcohol are produced by the fermentation of milk. This occurs most readily in milk containing relatively large proportions of milk sugar (such as sheep's, mare's, and camel's milk). The three principal forms will be briefly considered here.

Leben.—This was produced by the ancient Egyptians, Arabs, and Carthaginians from the milk of buffaloes, cows, and goats. It is usually prepared by boiling the fresh milk over a slow fire, after which some fermented milk from a previous preparation

is added to the warm article. The fermentation takes place rapidly and is considered to be complete at the end of six hours. The product is valued highly and is offered in hospitality to the passing stranger. In fact, in some parts of Arabia payment for this delicacy would be considered an insult.

The fermentation is due to the action of yeast and bacteria which produce both alcohol and acids. Some of the half-civilized tribes of Siberia prepare a strong alcoholic beverage by the distillation of leben. The distillate contains volatile acids, and from 7 to 8 per cent alcohol. The product is called leben, yoghurt, matzoon, gioddu or dadhi depending on the country in which it is made.

Kumiss.—This is the most famous of all fermented milks. It has been known from the most ancient times to the present day as the principal food of the wandering tribes who inhabit the steppes of European Russia and southern, western, and central Asia.

It is prepared by mixing one part of warm water with five parts of mare's milk. To this is added some old kumiss, and the mixture agitated until fermentation occurs.

The change undergone during fermentation consists of a vigorous production of gas and alcohol, accompanied by an acid formation which causes the coagulation of the milk. The coagulum exists in an extremely fine state of division, and the liquid froths violently when the bottle is opened. It is reported to have a full, pleasant acid taste and contains from 1 to 2 per cent alcohol. The fermentation is due to the combined action of bacteria and yeast. The comparative absence of pulmonary tuberculosis among the tribes which use this product in some cases has led to its exploitation as a cure for consumption.

Kefir.—Kefir is a kind of fermented milk which has long been used in the Caucasus. It differ from kumiss in that it is prepared from either sheep's, goats', or cows' milk. The fermentation is started by the addition of a few grains of kefir to the milk (which is contained in leather bottles). The kefir grains are small solid kernels which are kept in families and handed on from one generation to another. Traditions are prevalent among the Mohammedan tribes of the Caucasus that kefir grains were in the first instance presented by Allah to a preferred tribe as a sign of immortality. Others hold that, in past ages, they were found by shepherds growing on a shrub in the Caucasian highlands; whereas, according to Skolotowski, they were originally

found adhering to the walls of oaken vessels used in the preparation of airam, a sour milk beverage similar to kefir. They are a zooglea composed of bacilli and yeasts.

Other micro-organisms are known which produce the more complex alcohols—butyl and amyl alcohol. The latter is fusel oil and often causes poisoning when crude alcoholic fermentation products are consumed.

Acid Production.—Many micro-organisms decompose carbohydrates, sugars, starches, and celluloses with the production of acids. Ever since Pasteur showed that the production of acid is brought about by the action of micro-organisms, considerable attention has been given to this subject. This is due to the fact that (1) many of the acids produced are of commercial importance, and (2) the property of micro-organisms to produce various acids under definite conditions is so specific that the process may be used to identify various organisms.

The quantity and nature of the resulting acid depends upon the species of micro-organisms used and the media in which they are grown. Some organisms, as for instance the *aciduric* (acid-loving bacteria), not only produce and survive in the presence of considerable quantities of acid, but their proliferation is apparently dependent upon the utilization of carbohydrates or carbohydrate-like substances. Prominent members of this group are *Lactobacillus acidophilus* and *L. bulgaricus*.

There are other organisms which will grow either in a carbohydrate or carbohydrate-free medium, but the production of acid occurs only in the presence of a carbohydrate. The following are some interesting examples of this phenomenon: The diphtheria bacillus does not form its potent, soluble toxin in a glucose-protein medium, but produces lactic acid. In the presence of glucose the Shiga bacterium fails to manufacture its characteristic poison. It also produces lactic acid. Indol is not produced by the colon bacillus in the presence of a carbohydrate, nor does *Proteus vulgaris* produce its proteolytic enzyme under such conditions. Both produce acid products, mainly lactic acid. The organisms, in the presence of carbohydrates, get their energy from them; hence, carbohydrates become sparers of proteins, just as they do in the animal and human body.

The more important acids produced by bacteria are: Acetic, lactic, butyric, oxalic, propionic, hippuric, succinic, and formic. Only the first three will be given brief consideration.

Acetic Acid.—It has been known for a long time that when

beer, wine, and similar alcoholic liquors are left exposed to the air a tough, mucilaginous film or skin develops on their surfaces; the alcohol gradually disappears, and vinegar results. It has also been known that a small portion of this film, if transferred to nonfermenting liquors, causes them to ferment; hence, the substance has become known as mother of vinegar. Some shrewd guesses were made that the mother of vinegar was composed of living entities, but even as late as the last half of the nineteenth century, this was not the generally accepted idea. In 1864, that illustrious Frenchman, Louis Pasteur, proved that this fermentation is a physiologic process, the inception and maintenance of which is bound up with the vital activity of microorganisms to which he applied the name *Mycoderma aceti* (*Acetobacter aceti*).

Since that time many species of aerobic acetic-acid bacteria have been described. All of them are bacilli that usually grow in chains and do not produce spores. Some produce only small quantities of acid; hence, they have no commercial value. Others produce large quantities and are used in vinegar-making.

The acid results from the oxidation of the alcohol by an intracellular enzyme. This action proceeds best at a temperature of 20° to 30° C. in the presence of an abundance of oxygen and in a medium containing suitable nutrients. The greatest commercial value of these organisms is in the manufacture of vinegar.

Butyric Acid.—Fermentations which result in the production of butyric acid are common. Before Pasteur reported his work on acid fermentation, this condition was studied and reported by him as due to the action of bacteria. The organism causing butyric fermentation is now recognized as a bacillus, but Pasteur considered it to be an animal, for he found it actively motile. However, the phenomenon which especially attracted his attention was its ability to live without air. The organisms are anaerobes. Butyric fermentation is of importance chiefly because it imparts to food in which it has occurred a bad odor and flavor. Ordinarily, butyric-acid bacteria are inhibited from developing in food by the acetic or lactic acid present. However, the producers of these latter acids are easily destroyed by heat, whereas the butyric-acid bacteria form spores which may survive steaming for an hour and a half. They occur in the soil, and when they find their way into food products are hard to destroy. They sometimes cause the spoilage of canned goods; such as peas and beans.

Lactic Acid.—When milk is allowed to stand, it develops a sour taste and curdles. Investigations show that this flavor is due to lactic acid which is produced from a portion of the sugar, lactose, already present in the milk. The curdling comes from the precipitation of the milk casein by the lactic acid. This apparently spontaneous fermentation is the result of the vital activities of the bacteria which gain access to the milk after it is drawn from the cow; for if special precautions are taken to prevent their admittance or to destroy them by heat or other means after they have entered, the milk may be kept unchanged for an indefinite period.

Pasteur was the first to describe an organism characteristic of lactic fermentation and to prove the same capable of pro-

Fig. 49.—Microphotograph of preparation from Armenian soured milk (*After Douglas.*)

ducing acid in sweet, sterile milk. He named it the "ferment of lactic fermentation," and since that time the bacteria have been obtained and used in pure cultures. Many different species of bacteria are capable of producing lactic acid in milk, yet the principal ones responsible for the natural souring of milk belong to two groups—*Streptococcus lactis* and *Lactobacillus lactis.* A third group, the *Escherichia acidilactici,* yields gases and various other products in considerable quantities in addition to lactic acid.

To the producer and vender of fresh milk these organisms are objectionable, for at the proper temperature (30° to 35° C.), they quickly sour the milk. Hence, precautions are taken to prevent

the heavy seeding of the milk with these organisms, and it is kept cold to prevent their rapid multiplication. In the production of butter the lactic-acid bacteria play an important part in the ripening process to which cream is subjected before churning. At times, this fermentation is allowed to occur spontaneously depending upon the organisms which by chance find their way into the milk; at other times, buttermilk or sour milk is added to hasten the process. However, in the best factories today pure cultures of the lactic-acid bacteria are used as starters. This latter process insures a product of more uniform composition.

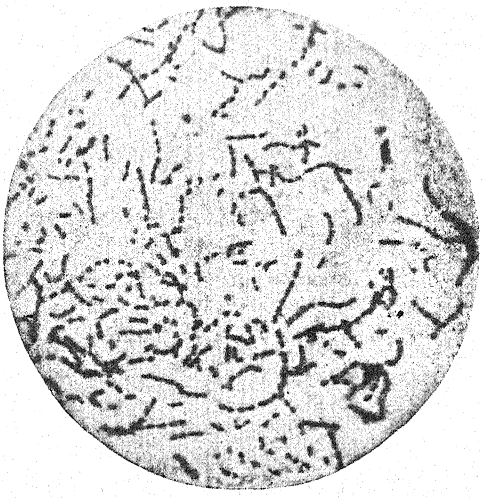


Fig. 50.—Streptococcus lactis. (*After Douglas.*)

Alkali Production.—In sugar-free media many bacteria produce an alkali. Among the principal ones formed is ammonia, which plays a leading rôle in the bacteriological changes occurring in soil. It is first produced as ammonia and later changed into nitrates. The production of alkali depends upon the micro-organisms bringing about the change and also the medium in which these micro-organisms grow. There may be production of an acid followed by that of an alkali. For example, milk when allowed to stand increases in acidity up to a certain concentration, after which the acid disappears. Later the milk becomes alkaline due to the production of ammonia by the breaking down of the casein.

CHAPTER XV

INFLUENCE OF ENVIRONMENT UPON BACTERIA

All living organisms are dependent upon certain environmental factors. Change the environment, and the organisms either perish or adjust themselves to the new conditions depending upon the specific organism, the magnitude, and the nature of the change. Micro-organisms are unique in that many of them withstand great environmental variations and apparently adapt themselves more quickly to new conditions than do the higher plants and animals.

Temperature.—The probable temperature limit at which bacterial life is possible is absolute zero (—273° C.) and 160° C. The temperature at which they can grow and carry on their other life activities is much narrower (0° and 90° C.). The rapidity with which they grow and function varies with the temperature. This is a universal law holding throughout the plant and animal kingdoms. Moreover, the rate of variation due to temperature is of the same magnitude as that occurring in chemical reactions in general. This principle is illustrated by experiments of Loeb and Northrop on the fruit fly. They hatched flies under aseptic conditions and fed them on sterile food in containers free from micro-organisms. These flies were grown at temperatures varying from 10° to 30° C. Those grown at 30° C. (which is not far from the temperature at which this species of flies normally live) had an average life of twenty-one days. At 20° C. they lived fifty-four and three-tenth days, or a little over twice as long. At 25° C. they lived thirty-eight and five-tenth days; and at 15° C. one hundred twenty-three and three-tenth days, or about three times as long. These investigators showed that the length of the fly's life is inversely proportional to the temperature at which the fly is kept, and that for each 10° C. drop in temperature the life of the fly is doubled or trebled. The flies kept at the lower temperatures moved about but were very sluggish (they lived slowly but for a long period). Those kept at the higher temperatures were very active. They lived fast and only a short time.

Bacteria at a low temperature multiply slowly and produce only small changes, but at higher temperatures their life activities are rapid. This is well illustrated in the case of meat. In cold storage it may be preserved for a year; in the warm kitchen it spoils in a few hours. In the first case, bacterial activity is slow; in the second, rapid.

There is for every micro-organism an optimum, minimum, and maximum growth temperature, as well as a thermal death point. The optimum temperature is the one best suited to the growth of the micro-organism. This varies widely with different species. Northern soils and cold waters contain numerous bacteria, the optimum temperature of which is 15° C. or even lower. Forester, in 1887, obtained from phosphorescent fish light-emitting bacteria. These micro-organisms not only lived at 0° C. but actually reproduced at this temperature. Since then numerous organisms have been isolated from soil and salt waters which multiply rapidly at 0° C. or slightly lower. To them has been given the name of *psychrophilic* or "cold-loving bacteria." Pigment-producing bacteria are especially abundant in this group. They are of considerable importance in soils of the northern climes, in the deep waters of oceans and lakes, and probably in the changes going on in meats and vegetables in cold storage.

In contrast to the psychrophilic bacteria are certain so-called *thermophilic* or "heat-loving bacteria." These organisms have been repeatedly obtained from the waters of hot springs, the interior of fermenting manure and ensilage, the soil, and the intestinal contents of man and animals. Some of them multiply at a temperature from 70° to 80° C. Setchell found some bacteria living in the water of springs the temperature of which was 89° C. What interesting specks of living protoplasm these must be to live and function at such temperatures. They must be composed of materials far different from those that make up the protoplasm of the ordinary plants and animals; for 89° C. is near the boiling point, in fact so high is this that it quickly coagulates egg albumen, produces painful burns on the skin, and kills most microbes.

Between the psychrophilic and thermophilic varieties is the group containing the overwhelming majority of bacteria—the *mesophilic* bacteria. These may be divided into two great classes: (1) Those found in the body of man and animals during health and disease. They have an optimum temperature of about

blood heat (37.5° C.). (2) The saprophytic organisms, many of which are essential soil organisms and have optimum temperatures between 20° and 35° C.

The minimum temperature is the lowest at which bacterial growth will occur. With many of the pathogens this is only 2 or 3 degrees below the temperature of the body of the animal which these specific bacteria attack. Some thermophilic bacteria have a minimum temperature as high as 40° C., and some psychrophilic as low as 0° C. or possibly even lower. The maximum temperature is the highest at which growth and multiplication can take place. This in the case of pathogens is only a few degrees above the optimum, but in the case of the saprophytes it may be many degrees above. For the thermophilic bacteria it may be as high as 89° C., whereas for the disease producers it lies between 40° and 50° C. Certain disease-producing organisms when grown for some time at a high temperature lose their disease-producing power but not their power of calling out antibodies; hence, this means is occasionally used in the preparation of vaccines. Most of the psychrophilic bacteria will not grow at a temperature above 30° C.

The temperature relations for the three classes of organisms are: (1) Psychrophilic micro-organisms—minimum at 0° C., optimum at 15° to 20° C., maximum at about 30° C. To this class belong many of the water micro-organisms, especially the phosphorescent bacteria in sea water, and many molds and yeasts. (2) Mesophilic micro-organisms reach their minimum temperature at 5° to 25° C., optimum about 37° C., and maximum at about 43° C. To this class belong all pathogenic bacteria, most parasitic, and many saprophytic micro-organisms. (3) Thermophilic micro-organisms have a minimum temperature ranging from 25° to 45° C., optimum from 50° to 55° C., and a maximum of 60° to 70° C. Some few are able to develop at a temperature of 80° C.

By carefully elevating or reducing the temperature, the limits within which certain bacteria will grow have been altered. The anthrax bacillus grows normally at 37° C. but under certain conditions it has been possible to gradually accustom it to a temperature of 43° C. Pigeons are naturally immune to anthrax, but if given strains accustomed to this high temperature they become infected. Likewise, frogs are immune to normal anthrax, but if given bacteria grown at 12° C., the frogs are killed.

The growth-temperature range for an organism is the number of degrees between the minimum and maximum. This is narrow for pathogens and wide for saprophytes.

Low Temperature.—Although bacteria cannot function below 0° C., they are still very resistant to low temperatures. The common soil and water organisms as well as the diphtheria and typhoid bacilli have been exposed for days to the temperature of liquid air (about —190° C.) without destroying them. Others have been exposed to the temperature of liquid hydrogen (about —250° C.) with the same results. At this temperature mercury becomes as brittle as cast iron. It is interesting to speculate on what would happen if these bacteria had been cooled down to —273° C. instead of —250° C. It does not seem probable that this relatively small difference would prevent them from recovering. At —273° C. (absolute zero) all chemical processes would come to a standstill; hence, if the cell would recover after exposure to this temperature we would have to conclude that cell life may be revived after all chemical activity has ceased within it.

Freezing causes a much greater decrease in the number of bacteria in water than it does in milk. Then too, alternate freezing and thawing is much more injurious than continuous freezing, as may be seen from the following results obtained by Hilliard and his co-workers:

CONTINUOUS FREEZING COMPARED WITH ALTERNATE FREEZING AND THAWING OF EBERTHELLA TYPHI

Frozen solid		Alternate freezing	
Before freezing	40,896	Before freezing	40,896
Frozen twenty-four hours	29,780	Frozen three times	90
Frozen three days	1,800	Frozen five times	0
Frozen four days	950	Frozen six times	0

Crystallization, probably resulting in the mechanical crushing of the bacteria, plays a prominent part in the death of bacteria due to freezing. Moreover, prolonged freezing causes a drying out of the cell, and we have seen that bacteria cannot continue to live in the absence of moisture. A very interesting condition is often found in natural ice filled with air bubbles. On examining the ice surrounding the air bubble it will be found to contain many more bacteria than does the rest of the block. Curiously

enough, these organisms are the oxygen-loving ones which have been attracted to this region by the air and then caught on the congealing of the water.

Thermal Death Point.—Thermal death point is often defined as that temperature which under given conditions will kill an organism in a given period of time. This is not strictly correct as it is well known that all the organisms in a culture when subjected to unfavorable conditions do not die instantly, but under definite standard conditions there is a definite rate of death. Consequently some authorities prefer the terms *thermal death rates* and *thermal death times*. It is perfectly evident that a living organism may withstand a comparatively high temperature for a short time, whereas a much lower temperature requires a considerably longer time in order to kill. The time, therefore, in determining the thermal death point is usually taken as ten minutes. One can place his hand in an oven that has a temperature of 130° C. for a short time, whereas if placed for this same time in steam (the temperature of which is 100° C.) a painful burn may be the result. The more effective action of moist heat is due to a number of factors: (1) Moist heat is more penetrating than dry heat; (2) the steam on changing to water liberates large quantities of heat; and (3) death is probably due to the coagulation of the proteins which compose the bacterial body. The temperature at which albumen coagulates varies with the quantity of water present, as may be seen from the following:

Egg albumen + 50 per cent of water coagulates at	56° C.
Egg albumen + 25 per cent of water coagulates at	74°– 80° C.
Egg albumen + 18 per cent of water coagulates at	80°–.90° C.
Egg albumen + 6 per cent of water coagulates at	145° C.
Egg albumen + 0 per cent of water decomposes at	160°–170° C.

Dry egg albumen may be heated to the point at which it decomposes without coagulation, consequently the thermal death point of micro-organisms in water would be much lower than for the same micro-organisms in dry air.

The housewife cans strawberries, raspberries, plums, and other acid fruits with considerable ease, but corn and peas often cause trouble. One is acid, the other neutral, and microbes are destroyed more easily in an acid or alkaline solution than in a neutral one. Hence, in determining the thermal death point of bacteria, a neutral salt solution is used.

Potatoes having on their surface resistant spore-bearing bacteria if dropped into boiling water and left for one hour may still be infected with bacteria. We find, therefore, that the temperature necessary to kill micro-organisms also varies with the species and the form in which it is when heated.

Bacteria which do not form spores and the vegetative form of the spore-bearers are as a rule killed by moist heat at temperatures of 65° to 70° C. The thermal death point for the cholera spirillum is 58° to 60° C.; for the typhoid bacillus 58° to 60° C.; for the vegetative form of the anthrax bacillus 60° C. and for the tubercle bacillus 60° C. If any of the conditions are varied the thermal death point changes. For instance in covered heated milk, the tubercle bacilli are destroyed in fifteen to twenty minutes at a temperature of 60° C. When not covered, a film forms on the surface. Such milk even after being heated for sixty minutes may have living tubercle bacilli in it. The spores of molds can withstand thirty minutes of dry heat at 100° C. but most of them when moist die below 100° C. Bacterial spores can even resist dry heat as great as 140° C., for a considerable length of time although moist heat of about 120° C. for twenty or thirty minutes is enough to kill any bacterial spore.

Light.—Light is essential to the life of higher plants and animals. In its presence plants manufacture their own food from carbon dioxide and water. In the presence of sunlight animals actually manufacture vitamin D from the provitamin ergosterol. However, the direct rays of sunlight may be injurious to living tissue. This fact is well known by all who have gone boating or fishing after long confinement indoors. Most bacteria are even more sensitive to light than are the cells of the human body; for diffused sunlight hinders their growth, and the direct rays of the sun are highly injurious. Pathogens are more sensitive than saprophytes. Some of the disease producers are killed almost instantly when exposed to the sun's direct rays. On this rests the foundation of the oft-quoted proverb: "Where the sun does not enter, the doctor does." The sunlight's effectiveness varies not only with the organism but with the specific light rays and the medium in which the micro-organisms are suspended. Only the ultraviolet, violet, and blue rays of the spectrum possess bactericidal action; green light has very much less power; red and yellow none at all. The germicidal power is greatest in (and some workers actually believe

confined to) the ultraviolet regions of the spectrum. These parts of the spectrum are more active against organisms in bouillon than in milk, and in a medium like soil the influence of the light is confined to the very surface. The action of light is probably due to chemical changes produced in the bacterial protoplasm or the medium in which the organism is suspended. There may even be a coagulation of the bacterial protoplasm. Bacteria grow poorly on a medium which has long been exposed

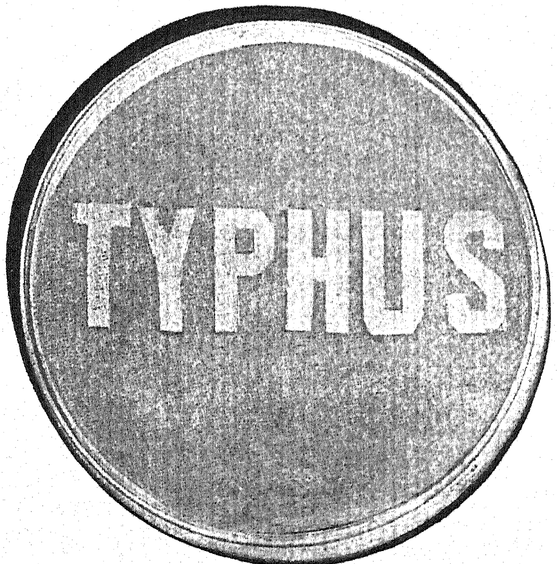


Fig. 51.—Thickly sown plate culture of *Eberthella typhosa* on agar-agar. Covered with paper letters and exposed to the sun's rays for one and a half hours, then kept twenty-four hours in the dark, whereupon development of thickly congregated whitish colonies was found only at the parts covered by letters. (*After H. Büchner.*)

to the action of light before seeding. This indicates that sunlight may produce toxic substances in and from the media.

The movement of some bacteria may be influenced by light. Engelmann found that if a microscopical preparation of *Rhodospirillum photometricum* be illuminated in such a manner that the light rays can fall only on one sharp definite portion, then, all of the roving bacteria collect within this space and move about briskly. If one of them in its onward career passes be-

yond the circle of illumination into the dark portion, it stops instantly and returns to the illuminated field. This Engelmann called the movement of alarm. Consequently, each sharply defined illuminated portion of the field acts as a trap for the bacteria, from which they cannot escape until the illumination has been altered.

Sunlight probably plays a major rôle in the sterilization of water and the destroying of pathogens which find their way into the air during coughing, speaking, and sneezing. Büchner, who was the first to appreciate the value of light in this respect, forcefully illustrated it by pouring bouillon agar inoculated with a copious supply of *Eberthella typhosa,* into Petri dishes on the underside of which were affixed the letters *TYPHUS,* cut out of black paper. The dishes were exposed to the sun's rays for a period lasting from one to one and a half hours (or to diffused sunlight for two hours), and afterward incubated in a dark room for twenty-four hours. On removing the paper letters, a growth of the bacteria which had been protected by the paper from the sunlight stood out boldly.

Other Rays.—x-Rays and radium emanations have little effect when allowed to play directly upon bacteria. However, when the bacteria are in living tissue they are often destroyed. This is probably due to a reaction of the stimulated tissue and not to the direct action of the various rays.

Electricity.—Can the microbe, like man, be shocked to death by the electric current? It would seem, from the work which has been done, that this is not possible. The passing of the electric current through a medium in which bacteria are growing destroys many of them, but this is not due to the direct action of the electricity, but rather to the heat generated and the chemicals produced in the medium as the current passes through it. The electric light kills bacteria if it is permitted to act on them a long time, but they are much more resistant to this than they are to sunlight.

Electricity is used quite extensively today in the sterilization of water and sewage, and some have suggested its use in sterilization of milk and other food products. However, the expense involved precludes its extensive use at the present time.

Electrophoresis.—Bacteria and colloidal particles when placed in water solutions take on an electric charge, the solution surrounding them becoming oppositely charged. If a suspension of bacteria be placed under the microscope and an electric field

be connected across the two ends of the slide, the bacteria will be seen to move toward the positive pole or anode. This phenomenon is called anaphoresis. If the particles are positively charged they move to the negative pole or cathode. This is known as cataphoresis. Now the phenomenon of migration of small particles under the influence of an electric current is electrophoresis. The charge acquired by bacteria in solution is probably due to a selective permeability of the cell membrane which lets more of the positively charged ions out than it does of the negatively charged ions or vice versa. The greater the charge on the cell the faster it will move in an electric field. Falk and co-workers found that virulent strains of pneumococci and diphtheria bacilli migrate in an electric field more slowly than do nonvirulent strains. This is probably due to the greater permeability of the cell membrane of the virulent strains, the toxins diffusing out more readily. Consequently, it can be used to determine virulence in micro-organisms and to a degree be used in place of the slower animal tests.

Shaking.—When subjected to prolonged shaking bacteria are first prevented from multiplication and later killed. This may be due to the actual breaking to pieces of the cell by the violent agitation. However, it is well known that continuous shaking of some proteins will cause them to coagulate.

Osmotic Pressure.—If a perfume is liberated in one part of a room its odor can soon be detected in any part. We thus find that gases tend to diffuse from points of high to points of low concentration. Similarly, if we dissolve copper sulphate in water, place it in the bottom of a tall cylinder, and cover this solution with pure water, we shall find, as time goes by, that the blue zone of copper sulphate creeps up into the clear solution and that eventually the two solutions will be of uniform concentration. The force which drives the copper sulphate from the point of high concentration to that of low is known as diffusion pressure, and is found to be dependent upon the number of particles of the substance in solution. By appropriate means this pressure can be measured and is the osmotic pressure. We have learned that a cell is covered by a membrane which readily allows water to permeate it but salts can do so only slowly Furthermore, we have learned that the concentration of protoplasm in the cell is about the same as a 0.9 per cent solution of common salt. A cell dropped into such a solution neither loses nor gains in weight, but if it is dropped into a more concentrated

salt solution it shrinks. A careful microscopical examination of such a cell shows that the inner ectoplasm has been torn from the cell wall. This is called plasmolysis. The salt solution bathing the cell is more concentrated than the solution found on the inside. The salt molecules cannot enter to equalize the pressure; hence, the water passes out through the cell membrane to dilute the solution. The plasmolysis of a cell may cause its death. It is because of these peculiarities that the placing of bacteria into strong salt or sugar solutions usually causes their death. Advantage is taken of this in the making of jellies, syrups, and preserves. Moreover, it is one of the factors which renders alkali soil barren. We find that when the osmotic pressure of the soil reaches about 8 atmospheres the activities of the nitrifiers are decreased, and are entirely stopped when the pressure has reached from 15 to 20 atmospheres. The ammonifiers are interfered with when the osmotic pressure reaches 9 atmospheres, but they are not entirely stopped in their actions until a pressure from 25 to 30 atmospheres has been attained. The nitrogen-fixing organisms are even more resistant to osmotic changes than are the ammonifiers.

Pressure.—Bacteria are extremely resistant to pressure, for they are found at depths in the ocean where the pressure is tremendous. However, successful attempts have been made to preserve fruit and vegetables by exposing them to high pressure. Apple juice subjected to a pressure of 4000 to 8000 atmospheres for thirty minutes did not develop gas later. Peaches and pears exposed to this pressure did not spoil for five years. However, those vegetables on which resistant spores occurred could not be preserved by pressure.

Reaction.—All living cells are influenced by the reaction of the medium in which they are living. Most bacteria require a neutral or slightly alkaline reaction for optimum growth, whereas yeasts and molds will tolerate and, in some cases, are actually favored by acidity. Hence, the microflora growing in any natural medium is governed by the reaction of that medium. Bacteria predominate in normal soils, whereas in acid or alkali soils there are a great number of molds. As lime or limestone is added to an acid soil and as an alkali soil is leached, the molds become fewer and the bacteria more numerous. Bacteria rapidly multiply in fresh milk until the acidity reaches a certain limit, after which they are replaced by molds.

Reaction, which in any medium is the relative acidity and

alkalinity, may be expressed in two ways: (1) By the number of cubic centimeters of normal acid or alkali required to bring the material to the neutral point of some indicator. This merely indicates the titratable acid or alkali and may or may not represent the neutral point, depending upon the specific indicator used and the medium being titrated. Proteins, for instance, may give a high titratable number and have a low acidity or alkalinity. In the past this has been the principal method used to determine acidity or alkalinity by the bacteriologist. (2) By the true hydrogen ion concentration, or conversely the true hydroxyl ion concentration of the medium. This may be determined by either conductivity methods or by the use of specific standardized indicators.

Acidity is determined by the presence of free hydrogen ions, and alkalinity by the presence of free hydroxyl ions in a given volume. A solution is truly neutral when it contains an equal number of hydrogen and hydroxyl ions. Pure distilled water is neutral, and analyses have shown that it contains approximately 0.000,000,1 Gm. of hydrogen ions in a liter. This may be written: 10^{-7} Gm. hydrogen ions per liter. A normal solution of hydrogen is one which contains 1 Gm. of hydrogen ions in 1 liter. Hence, a neutral solution is one which is 10^{-7} normal. Inasmuch as a neutral solution contains equal numbers of hydrogen and hydroxyl ions, it must also contain 10^{-7} equivalents of hydroxyl ions. The chemist has discovered that the normality of the hydrogen ion concentration times the normality of the hydroxyl ion of any solution is equal to a constant approximately 10^{-14}. Therefore, if the hydrogen or hydroxyl ion concentration of any substance be known, the unknown can be readily calculated. If the hydrogen ion concentration of a solution be 10^{-4}, the hydroxyl concentration would be 10^{-10}. Using this information we may make the following scale to indicate the acidity of solutions:

$$10^{0}, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7},$$
$$10^{-8}, 10^{-9}, 10^{-10}, 10^{-11}, 10^{-12}, 10^{-13}, 10^{-14}.$$

That is, the larger the numerical value of the exponent the smaller the hydrogen ion concentration and the greater the hydroxyl concentration. Such numbers are somewhat cumbersome; hence, Sörensen suggested that only the exponent be used, and this be taken as a positive number. This he designated the *p*H, hydro-

gen potential, of the solution. Written in terms of the pH values, our scale becomes:

0	8
1	9
2	10
3	11
4	12
5	13
6	14

7

All values to the left of the line indicate an acid solution, and the smaller the number the greater the acidity. All numbers to the right of the line indicate an alkaline solution and the larger the number the greater the alkalinity.

CHAPTER XVI

INFLUENCE OF CHEMICALS ON BACTERIA

During the infancy and childhood of surgery, pestilence followed in the path of the surgeon. Often the number of deaths occurring in a hospital corresponded with the number of patients who had entered. No wonder Simpson stated: "A man laid on the operating table is exposed to more chances of death than the English soldier on the field of Waterloo." Pasteur's discovery that fermentation and putrefaction are due to micro-organisms inspired Lister with a belief that the suppuration of wounds is due to microbes and that if their entrance into the wounds could be prevented this terrible slaughter would end. In order to accomplish this he attempted to disinfect doctor, nurse, patient, instruments, and even the air. Later it was learned that it is sufficient when those objects which come in direct contact with the wounds are sterilized. This discovery wrought such a transformation that today surgery is divided into two epochs—the pre-listerian and the listerian. The work of this pioneer was followed by the work of others, so that today disinfectants play a major rôle in the destruction of pathogenic micro-organisms; without them modern surgery and preventive medicine would be impossible.

Chemotaxis.—It has been repeatedly shown that bacteria, like other free-moving organisms, are attracted by certain chemicals in solutions and repelled by others. The attraction of a motile micro-organism by a chemical is *positive chemotaxis*, the repulsion being *negative chemotaxis*. A very simple and efficient method of studying this characteristic is as follows: A capillary tube 5 to 10 mm. in length and sealed at one end is filled with a 5 per cent slightly alkaline solution of peptone or beef tea. The outer surface of the glass is carefully cleaned from any trace of the medium and the tube is then placed in a drop of water containing bacteria. In a few seconds the bacteria thickly congregate around the open end of the tube. This is not due to volition on their part, nor is it due to their congregating in places most favorable for life; for had the capillary

tube been filled with sugar or glycerin, which are excellent foodstuffs, there would have been no such gathering of the bacteria at the end of the tube. Moreover, a dilute solution of potassium chloride and mercuric chloride attracts bacteria; but they rush into the tube only to meet their death. Some believe that bacteria are attracted to the roots of the alfalfa in the soil by some such means, and it is quite certain that this is the manner in which white corpuscles are attracted to an infected area.

Disinfectants.—Those substances which in minute quantities destroy the life of the cell are called poisons when considered in relation to man and animals; considered in relation to microorganisms they are called germicides. Analogous with the general term "germicide" are the terms "bactericide" and "fungicide." A disinfectant is a substance which destroys the causative agents of infection. It may imply sterilization, although in the majority of cases it does not. It implies the destruction of those minute forms of life which cause disease. The application of a disinfectant as a gas is termed "fumigation."

Antiseptics prevent decomposition and decay. They do not necessarily destroy micro-organisms, they prevent their growth and activity. A substance may be a disinfectant under one condition and an antiseptic under another. Formaldehyde in the proportion of 1 to 50,000 may be an antiseptic whereas it requires from 3 to 10 per cent solutions to become disinfectants in a reasonably short time. Mercuric chloride, in the proportion of 1 to 300,000 sometimes will prevent germination of anthrax spores, yet it requires 1 to 1000 solution to kill them.

The term "preservative" is generally applied to substances which are added to foods or feeding stuffs in order to prevent decomposition or decay. They may be either the nontoxic compounds sugar or common salt, or the slightly toxic substances, benzoic acid, boric acid, salicylic acid, formalin, or sulphites. The method of action of the two is very different: The first increases osmotic pressure and the second combines with the protoplasm of the cell.

Deodorants are substances which mask or destroy unpleasant odors arising from putrefying or fermentation of organic matter. A deodorant may or may not be a disinfectant. Formaldehyde is a good disinfectant and deodorant, whereas charcoal is a good deodorant but has no value as a disinfectant.

Classes of Disinfectants.—The classification of disinfectants is difficult inasmuch as in many cases we do not understand their

complete mode of action. Almost any compound, if used in sufficient concentration, may act as an antiseptic if not as a disinfectant. They may be classified from a physical, chemical, or even a physiologic standpoint. From the physical standpoint they may be grouped into the convenient, although very unscientific, classes known as solids, liquids, and gases. Chemically viewed, we recognize acids, alkalis, metallic salts, hydrocarbons, alcohols, aldehydes, anesthetics, essential oil, and oxidizing and reducing agents. The first three (acids, alkalis, and salts) are distinguished from the rest by being electrolytes. The strength of acids and alkalis depends upon the pH concentration, whereas that of metallic salts is dependent upon the nature of the metallic ion and upon the degree of ionization.

Rosenau classifies disinfectants according to mode of action as follows: (1) Those compounds which destroy by oxidation as ozone, chlorinated lime, potassium permanganate, and the halogens; (2) those which destroy by ionic poisoning with coagulation, as the metallic salts, mercury, silver, lead, and copper; (3) those which destroy by coagulation and poisoning, not ionic in character, as carbolic acid and its derivatives, and (4) those which destroy by emulsoid action, that is, through brownian movement and adsorption, as soap solutions and creolin.

Characteristics of a Good Disinfectant.—The ideal disinfectant is not known and may not be discovered, as a good disinfectant against one germ and under one condition may be extremely poor for another germ or under different conditions. Nevertheless, there are certain characteristics which must be met by a useful disinfectant. These have been condensed from Dreyfus as follows:

1. *High Germicidal Power.*—In order to list it as a good disinfectant, a chemical must have appreciable germicidal power. A chemical may be a good disinfectant for one bacterium and inactive toward another. This is one reason why it is impossible to make specific statements concerning the general utility of disinfectants. In many cases disinfectants show a marked specificity for certain bacteria.

2. *Stability.*—The disinfectant must be fairly stable and not have a marked affinity for organic matter. If it does react readily with organic matter, it will not be available for action on the bacteria. Such a disinfectant would have to be used in great excess to satisfy the demands of the organic matter and to leave sufficient to function as a bactericide. It is easily un-

derstood that a compound, which in large amounts may be an active disinfectant, may be so reduced by organic material that it becomes merely a preservative or antiseptic. A good disinfectant should be stable and not undergo spontaneous decomposition, as is exemplified by hydrogen peroxide.

3. *Readily Soluble in the Strength Required for Disinfection.*—Before a chemical can function as a disinfectant, it must, of necessity, be soluble in some material such as water or alcohol. It is not necessary that it be extremely soluble, but only in concentrations sufficient to function as an active disinfectant. Under certain conditions mercuric chloride is an active disinfectant. Mercurous chloride, however, cannot be used as a disinfectant on account of its insolubility in water.

4. *Nonpoisonous to Animal Life.*—The ideal disinfectant is one which is not poisonous to higher animals in the concentrations used for disinfection. It is difficult to select a compound not harmful to the tissues yet which will destroy the parasite. This is well illustrated by the disinfectants which have been used for such infection as sore throats. Many of these are worthless in the concentrations used and could not be used in higher concentrations; otherwise, they would attack the tissue in the throat and make it more susceptible to infection.

5. *Noncorrosive.*—Corrosive chemicals are extremely difficult to store and their strength may be greatly reduced by their action on the containers. Quite often such compounds are diluted as much as possible to avoid their corrosive action.

6. *Sufficient Power of Penetration.*—Disinfectants are often needed for the deeper layers of the tissue, and it is obvious that their application to the surface may be useless. Few compounds have good penetrating powers, and physicians and surgeons find it necessary to apply the disinfectant in the immediate vicinity where it is needed. To illustrate, we have had numerous pharmaceutical products prepared for the treatment of pyorrhea. This infection is a deeply seated abscess at the base of the teeth, and it is absurd to expect a disinfectant incorporated in a dentifrice to destroy the bacteria in such abscesses.

7. *Moderate in Cost.*—Cost cannot be considered by itself. It must be considered along with the germicidal powers of the compounds. However, it is usually the second prerequisite that enters into the selection of the disinfectant—first, activity and second, cost. The selection of a disinfectant with regard to cost is often determined by the amount that will have to be used.

Rules Governing the Action of Disinfectants.—When using disinfectants, there are certain fundamentals which should be observed. They may be summarized as follows:

1. The efficiency of a disinfectant varies with the amount of moisture present. A dry poison has slight action on bacteria. Dry formaldehyde, or sulphur dioxide, is practically without effect. In a similar manner absolute alcohol has not nearly the germicidal power of 60 to 70 per cent alcohol. This is probably due to the coagulation of the outer membrane of the organism by the absolute alcohol, thereby preventing the poison from diffusing into the vital parts.

It is obvious that the efficiency of a disinfectant varies with its concentration, but it is not always the same for different disinfectants. Doubling the concentration of mercuric chloride in water halves the time necessary for sterilization; doubling the concentration of phenol diminishes the time about sixty-four times.

2. The temperature of the medium in which the organisms are present materially influences the action of a disinfectant Some idea of the magnitude of the effect of temperature may be gained from the fact that with metallic salts the mean velocity of disinfection increases two- to four-fold for a rise in temperature of 10° C., whereas with phenol it is as high as eight-fold, using *Salmonella paratyphi* as the test organism. Hence, the use of a disinfectant at a comparatively high temperature, other things being equal, is more effective than its use at a low temperature. In reality, a solution which at one temperature is only an antiseptic may become a disinfectant by a small increase in temperature.

3. The power of a disinfectant to kill bacteria is dependent in a remarkable degree upon the nature of the medium in which bacteria are present when the germicide is applied. Almost invariably the greatest germicidal activity is shown when the substances act upon the bacteria freed from all contaminating culture media and suspended in distilled water or salt solution. The presence of proteins and similar substances usually causes a great reduction in the germicidal powers of the substance. If the protein is a good nutrient medium, the micro-organisms are even more resistant. Bacteria are highly resistant in the presence of pus, many of the organisms being in the body of dead leukocytes.

This decreased efficiency in the presence of a protein is vari-

ously explained. In the case of such disinfectants as phenol and the dyestuffs, it is frequently stated that the disinfectant is "quenched" or "fixed" by the protein medium. Adsorption in some cases may play a part, but the salts of heavy metals, combined with the protein-yielding insoluble nonionizing proteinates. The low germicidal action exhibited by most antiseptics against pus is due in part no doubt to the mechanical difficulties of penetrating the particles in the pus.

4. Emulsion may have greater germicidal power than true solutions. Emulsions are mixtures having small globules of the concentrated poison distributed throughout the solution; if examined under the microscope, these, together with the suspended bacteria, will be seen moving about in the solution. When bacteria enter one of these highly concentrated drops of poison they are readily killed. Larger objects which may be in the suspension due to adsorption concentrate it at the surface; hence, the strength of emulsions is rapidly reduced. The value of phenol is scarcely impaired by the presence of organic matter in suspension, whereas emulsified disinfectants are reduced to one third or even one half their original value.

5. Disinfectants of the heavy metal group, in order to be effective, must be in solution so that ions are produced which combine with the protein of the bacterial cell and thus cause death.

6. Disinfection by nearly all germicides proceeds more rapidly in an acid than in an alkaline medium. Pure alkalis and soaps which act by virtue of their alkali content are the two principal exceptions.

7. The effect of salts in the medium depends on their nature, the disinfectant, and their concentration. In general, salts increase the action of phenols and of emulsified disinfectants but diminish that of mercuric chloride.

8. The action of disinfectants varies with the time. Even a fairly weak poison acting for a long time will ultimately cause death.

9. The effectiveness of disinfectants varies with the number of organisms in the medium to be sterilized. Highly infected material is more difficult to sterilize than slightly infected.

10. Substances which reduce the surface tension of the medium carrying the bacteria tend to accelerate the action of the disinfectant. It has been demonstrated that hexylresorcinol, when taken by mouth in therapeutic doses, is excreted in the urine in

sufficient concentration to kill bacteria and that the simultaneous administration of sodium carbonate robs the urine of its bactericidal power. Neither the alkalinity of the urine nor the presence of the soda has any direct effect on the activity of the hexylresorcinol. However, it was found that the administration of hexylresorcinol in therapeutic doses resulted in the secretion of urine of very low surface tension, whereas the administration of soda gives rise to urine of very high surface tension. These observations suggested that surface tension may play a part in the activity of some disinfectants, and Frobisher found a direct relationship between bactericidal power and ability to lower surface tension. However, this must not be taken to imply that substances like soaps, sugar, or peptone, showing no chemical germicidal action, can be made germicidal merely by reduction of the surface tension of the solution. It would appear that if a substance has a slight tendency to destroy bacteria by reacting with them chemically, the changing of surface tension may accelerate or retard, depending upon whether surface tension is lowered or increased. This may come from solutions of low surface tensions concentrating at the surface and entering minute crevices which may hold back solutions of high surface tensions. Hence, the chemical may pass into the cell and do damage which otherwise would be impossible, were the surface tension high.

11. Young cultures of bacteria are usually more resistant than are older cultures, provided they have not formed spores. Spores are more resistant than are vegetative bacteria.

12. Bacteria possessing a high lipid content, such as the acid-fast bacilli, are extremely resistant to liquid disinfectants. Tubercle bacilli withstand 5 per cent phenol for over twenty-four hours but are killed in one minute by boiling. Consequently, for the destruction of spores and acid-fast bacteria, heat is preferable to chemical disinfectants.

Standardization of Disinfectants.—It is important to have methods by which the disinfectant value of various chemicals may be measured. Koch seeded suitable micro-organisms, such as anthrax spores, onto silk thread; after exposing to varying concentrations of the disinfectant they were washed and transferred to suitable culture media. The disinfectant remaining on the thread retarded germination; hence, Krönig and Paul replaced the thread with garnets and plated the washings from the garnets. Later these methods were replaced by the Rideal-Walker drop method and the Chick-Martin test. The former,

in some of its various modifications, is quite generally used today. It measures the efficiency of various disinfectants in terms of an adopted standard, phenol; consequently, the strength of a disinfectant is referred to as the phenol coefficient. This is determined under standard conditions; test organism, medium, temperature, and time. Generally, *Eberthella typhosa* (Hopkins' strain) is used as the test organism, although any organism may be used, provided it is so stated. The phenol coefficient for staphylococci and typhoid bacilli are far different. There may be a wide variation even among strains of the same species. A low coefficient indicates a weak germicide and a useless disinfectant, but a high coefficient is not necessarily a true indication of a favorable agent in practical work. It must meet the characteristics given for a good disinfectant. These factors can be determined only after careful testing and long practical use. Hence, while many new and probably highly efficient disinfectants are being developed, one should be cautious in replacing those which have stood the test of time, mercuric chloride, carbolic acid, cresols, lime, hypochlorites, chlorine, formaldehyde, and iodine, with new untested disinfectants.

Use of Disinfectants.—It is essential to know what, how, where, and when to use disinfectants. They are used against very small living entities and special attention must be given to the minutest details; otherwise, time and money are wasted and a false sense of security is created. The nature of the infection, the infected material, the strength of the disinfectant, the mode of application, and the effect of temperature and humidity must all be considered.

It is evident that all substances carrying pathogens should be so treated that they will not convey to man or the lower animals infection. The means selected must vary with the infection. However, disinfectants should never be made to take the place of cleanliness. If possible, infection should be prevented, but when an object becomes infected the infection should be removed or destroyed. It is quite generally known that wounds should be disinfected at once, but it is not so generally known that the same principle should be applied in the case of all communicable diseases. In the past terminal fumigation played a leading rôle in disease prevention. Today the golden rule is to disinfect immediately all objects which may carry the pathogens —discharges from the body, handkerchiefs, towels, clothing, bedding, food, tableware, and other objects which have been used by the ill in eating and in drinking. Nor should the hands of

nurses, physicians, and others who have come in contact with the infection be overlooked.

Chemical disinfectants should never be made to take the place of soap, water, and the scrubbing brush. Not that these kill germs, but many germs are removed by the soap and water and others are exposed directly to the disinfectant which follows. Wounds filled with dirt are not ready to be disinfected until all foreign material has been removed. Milk cans coated with grease and other foreign material may be treated with various chlorine solutions and still carry many undesirable microorganisms. The same cans after being carefully cleaned with hot water and washing powder, are readily sterilized by chlorine, steam, or other means. An advanced student working in our laboratory found that drinking glasses, after being dipped into B. K. solution, usually carried contaminates from the mouth, whereas if first washed with soap and hot water and then dipped into B. K. solution they are no longer a source of danger. Hence, the watchword in sanitation should be cleanliness, clean food, clean milk, clean water, clean hands, clean rooms. These, at times, may require physical or chemical treatment to render them safe. Yet clean food, person, and environment are always to be preferred to those laden with filth, even though they carry no living germs.

Mercuric Chloride as a Disinfectant.—Mercuric chloride is one of the best, most effective, and most generally used of the metallic salt disinfectants. It will kill some varieties of bacteria in a few minutes when present in a dilution of 1 to 50,000. It inhibits the growth of most bacteria when present in solution in 1 to 10,000 parts of water. A solution of 1 to 1000 is ample for the destruction of all nonspore-bearing bacteria, provided it comes in direct contact with the bacteria for sufficient time. Most spores are killed in a 1-to-500 water solution in one hour. It is most effective in water solutions and its efficiency is greatly reduced in alkaline solutions containing proteins, which precipitate out the mercury leaving it ineffective. Hence, it is not suitable in fluids containing blood, blood serum, pus, sputum, and body fluids.

For ordinary purposes, it is employed in concentrations of 1 to 500 and 1 to 2000, both of which will kill the vegetative forms of bacteria in from one to twenty minutes. The stronger solutions are used when much organic matter is present. It is especially valuable for disinfecting the hands and for washing floors,

woodwork, and furniture. It attacks metals and hence cannot be used for their disinfection. Following its use, the objects should be thoroughly washed. It is odorless, and in order to avoid accidents its solution should always be colored with some dye.

Carbolic Acid.—Crude carbolic acid is a mixture of a number of compounds and has a phenol coefficient of 2.75; hence, it has a higher germicidal efficiency than phenol (the coefficient of which is 1). A solution of 1 to 1000 parts inhibits the growth of bacteria, 1 to 400 parts kills the less resistant bacteria, and 1 to 100 parts kill all nonspore forms. A 5 per cent solution kills the less resistant spores within a few hours and the more resistant in from one day to four weeks. Efficiency is greatly increased by an increase in temperature.

It is a highly useful disinfectant with a wide range of uses. It is not destructive to fabrics, colors, metals, or wood and is not impaired by proteins to the same degree as is mercuric chloride. Its disadvantages are that it has a penetrating odor, an acid taste, and is a corrosive poison. It is inactivated by alcohol; hence, burns due to carbolic acid should be treated immediately with alcohol.

Cresol, Creolin, and Lysol.—The cresols are about three times as efficient as carbolic acid. Creolin and lysol are prepared by emulsifying cresols in soap solutions. Although their efficiency is decreased by organic matter, it is not decreased to so great an extent as in the case with many disinfectants. Creolin has a phenol coefficient of 3.25 without organic matter and 2.52 with organic matter. Lysol has a phenol coefficient of 2.12 without organic matter and 1.87 with organic matter. Their uses are practically the same as for carbolic acid.

Disinfectants of the Chlorine Group.—To this group belong many of the more active disinfectants. They are all characterized by a chemical instability in the presence of organic matter. The members of this group contain active chlorine in distinction to inert chlorine, such as that in common salt. The phrase "active chlorine" does not necessarily imply that free chlorine is contained in the substance or is liberated by it. The active agent may be hypochlorous acid or some other compound containing chlorine. Their uses range all the way from the disinfecting of sewage to the disinfecting of human wounds.

Chlorinated lime or bleaching powder is extensively used in disinfection of sewage, outhouses, and of cellars, as well as for

other miscellaneous purposes. From 1908 to 1912 it was extensively used in water purification. In practice, from 5 to 12 pounds of bleaching powder is used to each million gallon of water. Since 1912 chlorinated lime has been rapidly replaced by liquid chlorine. The quantity added is from 0.25 to 1 part per million. By this method polluted waters are rendered safe. When used in waters containing considerable organic matter it may give rise to compounds with unpleasant flavors. The method, however, is cheap, reliable, efficient, harmless, and easy of application.

Within recent years chlorine-yielding compounds such as Dakin's solution and mild organic derivatives of hypochlorous acid have been extensively used for the cleansing of wounds and for the irrigating of infected tissue. They were especially helpful during the World War, when thousands of soldiers came back from the firing line in a horribly mangled condition with infected wounds; undoubtedly, thousands both in military and civilian life who would otherwise have fallen prey to infection have been saved by their use.

Iodine.—In 1909 Stretton introduced the use of iodine as a skin disinfectant. Since then it has been generally used for the sterilization of the skin, before operations, for hypodermic injections, for disinfecting wounds, and in infections of accessible mucosa of the nose and throat. It was widely used on wounds during the World War, but according to Dakin is far too irritating for repeated application. It is useful on superficial wounds where rapid and complete disinfection can be obtained by a single application. It has a greater penetrating power than most disinfectants. A 2.5 per cent solution is usually strong enough and 70 per cent pure alcohol is the best solvent.

Hydrocyanic Acid.—Hydrocyanic acid is a powerful insecticide but a poor germicide. It is used rather extensively against mosquitoes, lice, bedbugs, and cockroaches. It is effective against bacteria no hardier than diphtheria and typhoid, but it cannot be depended upon as a general disinfectant. Because of its highly poisonous nature, it must be used with extreme caution.

Formaldehyde.—Formaldehyde is one of the best volatile antiseptics. If used in sufficient concentration and under proper conditions it can be depended upon for surface disinfection. Although more penetrating than sulphur dioxide, it is not sufficient to depend upon in deep layers of clothing. It does not rot nor bleach fabric nor tarnish metal as does sulphur dioxide. More-

over, formaldehyde unites with nitrogenous substances, forming new chemical compounds which are both sterile and odorless.

Although there are numerous methods recommended for the liberation of formaldehyde gas, one of the best is that recommended by the Pennsylvania Department of Public Health:

	Ounces.
Sodium dichromate	10
Formalin	16
Commercial sulphuric acid	$1\frac{1}{2}$

The sulphuric acid is added to the formalin and the mixture poured over the crystals of sodium dichromate, causing immediate liberation of formaldehyde gas. Five hundred cc. of formalin and 250 Gm. of sodium dichromate should be used for each 1000 cubic feet of air. The floor should be protected against the heat by placing the bucket upon a brick or other suitable device.

The results of numerous experiments have demonstrated that the presence in the air of 2.5 per cent by volume of the aqueous solution, or 1 per cent by volume of the gas, is sufficient to destroy within a few minutes fresh virulent cultures of common nonspore-bearing pathogens.

Stahl has shown that bandages and iodoform gauze can be kept well sterilized by placing in the jar tablets of paraformaldehyde containing 50 per cent of formaldehyde. He also succeeded in freeing carpets and clothing of germs in from fifteen to twenty minutes, without in any way injuring the fabric, by spraying with a 0.5 to 2 per cent formaldehyde solution. A 2 per cent water solution of formalin is effective against nonspore-forming bacteria in about five minutes.

Sulphur Dioxide.—Sulphur dioxide is not especially effective as a germicide; it is, however, an effective insecticide. Hence, it is quite generally used in diseases spread by rats, mice, fleas, mosquitoes, and bedbugs.

Its action as a germicide depends upon the presence of moisture. The dry gas is practically without effect against bacteria. It cannot be depended upon where penetration is required, its action being merely upon the surface. It does not kill spores; moreover, it is a bleaching agent and tarnishes metals. In sterilization, by means of sulphur, time is an important factor. The things to be disinfected should be exposed for eight hours in an atmosphere of at least 4 per cent by volume of sulphur dioxide in the presence of moisture. This requires the burning of 4 to 5 pounds of

sulphur for every 1000 cubic feet of air space. About ⅕ pound of water should be volatilized for every pound of sulphur burned.

One method of using it is as follows: The required quantity of sulphur is placed in a pan which is placed in a second larger pan containing water. The sulphur is made into little craters and liberally soaked with alcohol and ignited. It is well to place the generator on a table or box, as sulphur dioxide is heavier than air and tends to sink and if placed on the floor may extinguish the flame.

CHAPTER XVII

THE CARBON CYCLE

MATTER is indestructible; it can be neither generated nor destroyed by any means known to man. He can change it from one compound into another, and varied are the forms in which he can make it appear. It has existed since the birth of the world and the rôles which it has played have been numerous indeed. The same bit of phosphorus, carbon, nitrogen, or other chemical element has appeared at one time in the apparently indestructible rocks of the everlasting hills, a little later in the body of a plant, and then in the body of a lower animal. Still later this same bit of matter may have formed a vital part of the body of a man. Plant, animal, or man may have been devoured by bacteria, and this same bit of material was returned to the soil to commence once more its wonderful journey through the bodies of plants and animals. In these transformations from the complex to the simple, from the simple to the complex, from the living to the dead, and from the dead to the living, bacteria play a very prominent rôle.

Carbon.—The element carbon is well represented in hard coal. Soft coal and charcoal are chiefly carbon. The diamond is pure crystallized carbon, and charcoal made from pure sugar is pure uncrystallized carbon. Carbon has certain outstanding properties; it has a valence of four and readily combines with itself and other elements, thus yielding a great variety of interesting and valuable compounds. There are at the present time approximately 250,000 compounds of carbon known, and the possibility of new compounds is limitless. Moreover, "carbon is the element par excellence, whose absence from any chemical substance stamps this forthwith, by common if somewhat arbitrary consent, as inorganic: Whose presence affords the soil and season for the growth of what might be termed 'the tropical jungle in the domain of chemistry.' For in the compounds of carbon nature seems to have run riot, in a revel of creative versatility, as if trying to set a record unapproached elsewhere in all the realm of

chemistry, for number, variety, and complexity of her children. Other elements—oxygen, nitrogen, phosphorus, sulphur, iron—indeed play a significant rôle in life processes; but the indispensable bond that ever links all other ingredients in organic unity is carbon. Furthermore, carbon is preëminently the energy carrier, the standard coin of the organic realm, for in it both the first cost of installation, of anabolic tissue building, and also the running cost of operation, of metabolism, is defrayed."

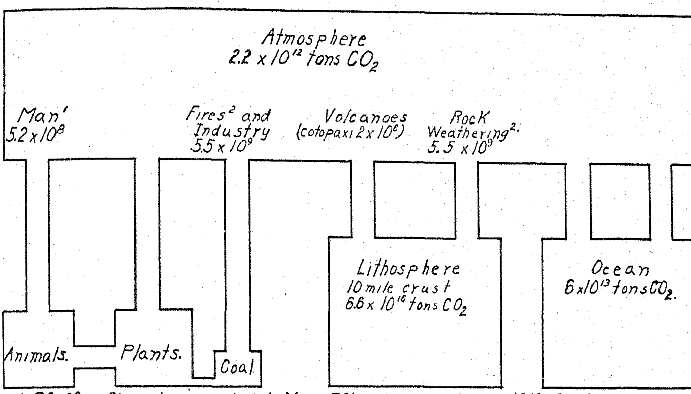
Occurrence of Carbon.—Carbon is widely distributed in nature. It is found in the earth in a pure form as diamonds and graphite. It occurs in the earth as coal to the extent of over 500,000,000,000 tons. Chemically combined, it exists in far larger quantities in limestone, chalk, marble and dolomite. It is estimated that 0.19 per cent of the entire earth is carbon and that sedimentary rock alone contains 30,000 times as much as occurs today in the atmosphere. The atmosphere contains about 3 parts in 10,000, which is equivalent to 600,000,000,000 tons of carbon. There is as much more in living things as there is in the atmosphere, and according to Clark from eighteen to thirty-seven times this quantity in the ocean. According to Pettenkofer, a man weighing 154 pounds contains 26.4 pounds of carbon; thus, no less than 257,000,000 tons of it is stored up in the bodies of men and women living upon the earth at the present time, in addition to the far greater quantities occurring in the tissues of plants and lower animals.

While the earth was in the molten state carbon occurred in the atmosphere as carbon dioxide. As the earth cooled this was absorbed and when the temperature dropped so that water could exist upon it, water also drank in the carbon dioxide from the atmosphere. The huge primitive plants which covered this earth in early geological ages were potent factors in removing it from the atmosphere, and as the carbon dioxide of the atmosphere decreased climatic changes occurred, until there resulted our present condition.

The Cycle of Carbon.—There are two great factors at work removing carbon from the atmosphere: (1) The decomposition of carbon dioxide by plants with the liberation of oxygen and the production of organic compounds; (2) the consumption of carbon dioxide in the weathering of rocks. No precise valuation can be given to either of these factors, although various attempts have been made to estimate their magnitude. Cook considers that leaf action alone more than compensates for the produc-

tion of carbon dioxide. Chamberlain estimates that the amount of carbon dioxide annually withdrawn from the atmosphere is 1,620,000,000 tons, the greater part of which is taken up by the weathering of rocks.

It is being continually added to the atmosphere by several processes: The combustion of fuel, the respiration of animals, and the decay of organic matter. Enormous quantities are being evolved from mineral springs and volcanoes. The magnitude of this factor can be seen in the statement "that if volcanoes became extinct it would not be long before the existence of life would be impossible due to a lack of carbon dioxide." Krogh



1. Pfeffer Physiologie quoted by Yost Pflanzenphysiologie 1913 Pg 151.
2. F. W. Clarke, Data of Geochemistry 1920, Pg 49.
Figures Without Denomination are Metric tons CO_2 per Annum.
Figures With Denomination are Metric tons Actually Present.

Fig. 52.—Illustrating carbon cycle in nature. (*After Lotka.*)

estimates that the annual consumption of coal adds yearly to the atmosphere about one thousandth of its present carbon dioxide content. Hence, there are two compensating sets of factors continually at work in nature: (1) Plant growth and rock weathering which is taking carbon dioxide and building from it more complex compounds. (2) Decay, respiration and combustion which are continually returning it to the atmosphere. These two sets of processes nearly balance each other, thereby completing the carbon cycle and rendering the carbon dioxide content of the atmosphere nearly constant.

Carbonation, which is the union of carbon dioxide with a base, plays an important part in the removal of carbon dioxide from

the atmosphere. A familiar example of this is the hardening of plaster due to the combination of lime with carbon dioxide and the formation of limestone. In nature many minerals take up carbon dioxide. This changes their volume and often their solubility, and as a result the rocks fall to pieces. This process increases the solubility of the potassium and the phosphorus of the soil. Högbom stresses the importance of the carbonation process by stating that it nearly balances the regeneration of carbon dioxide resulting from the burning of coal. Its accumulated record is found in the sedimentary rocks which contain approximately 30,000 times as much carbon dioxide as is today found in the atmosphere.

Photosynthesis.—Plants absorb water from the soil by means of their roots. This passes upward through the stem, the petiole, and the veins, and thence enters the main portion of the leaf. Here it comes in contact with carbon dioxide which has been taken in from the air. Although the quantity in the atmosphere is only three parts in ten thousand, yet it is effectively picked out by the green leaves, as a single large tree may expose a leaf surface of more than an acre, and the surface which absorbs carbon dioxide is much greater than this. In the presence of chlorophyll and under the influence of sunlight the water is caused to combine with carbon dioxide. Oxygen is given off. Probably the first product formed is formaldehyde. This is a poison and is not permitted to accumulate. The first product which does accumulate in sufficient quantities to be detected is glucose or grape sugar. This is present in the sap of practically all plants, and ultimately from it are produced all the varied organic compounds of the plant and animal kingdoms. The process of photosynthesis is fundamental in organic nature, for not only is it an essential function of green plants, but it is vital to animals and man; because (with the exception of the small quantity of carbon fixed by a few species of bacteria) it constitutes the source of food for all living organisms.

Attempts have been made to compute the quantity of carbon fixed by plants, but these can only be taken as approximations. One of the earliest attempts was made by Liebig. He considered the annual output of dry organic matter of central Europe to be 1 ton per acre. If 40 per cent of this were carbon, there would be an annual total production, by plants, of 13,000 million tons of carbon. This is nearly ten times the world's annual coal consumption and about one fifteenth of the total carbon

in the atmosphere. The botanist, Yost, computes that if the entire land area were planted to sunflowers, approximately 6.5×10^{11} tons of carbon dioxide would be annually absorbed; whereas, Arrhenius points out that if all the carbon annually fixed by plants were deposited in peat bogs, the atmosphere would be depleted in half a century. However, this does not occur, as there are many factors liberating carbon dioxide.

Production of Carbon Dioxide.—Probably one of the greatest sources of atmospheric carbon dioxide is from volcanoes and mineral springs. Cotopaxi alone has been credited with an annual discharge of 2,000,000 tons of the gas.

From every chimney of every factory and home where gas, oil, wood, or coal is burned, carbon dioxide is being returned to the atmosphere. The breath of every living animal is laden with it. In reality, all the coal that is mined, all the trees that are cut for fuel, all the food that is used for energy by man and the lower animals pass into the atmosphere as carbon dioxide. The magnitude of each can be only roughly measured. It is estimated that from coal fires and metallurgical furnaces alone ten times as much carbon dioxide is returned to the atmosphere annually as by the biological process of breathing. Man alone in the course of five hundred years would throw off enough carbon dioxide to double that in the atmosphere were there no compensating factors removing it. The forests are being burned and the deposits of peat and coal evaporated. During one season huge quantities of carbon dioxide are poured into the atmosphere. During another season equally large quantities are removed; yet, the carbon dioxide content of the atmosphere remains nearly constant. This is due to the ocean acting as a great buffer, at one time giving off, at another time drinking in carbon dioxide. Thus of every 2000 pounds of carbon dioxide thrown into the air by volcanoes, fires, and animal respiration, the ocean ultimately receives directly or indirectly about 1900 pounds, leaving only the balance of 100 pounds in the atmosphere.

As outlined above, there are certain leaks in the carbon cycle, and were this the entire process, the carbon in the bodies of plants and animals, which are not consumed by fire, would be permanently locked up. But this is not all. Plants and animals (including man) are permitted to keep their bodies as long as they are useful, but just as soon as life becomes extinct they are dragged off to the bacterial bonfire, so that the elements composing them can serve other plants and animals.

Decay.—During the process of decay, carbon and hydrogen are liberated as water, carbon dioxide, methane, and other volatile products, with the result that the carbon in the medium tends to decrease relatively to the nitrogen; hence, the carbon-nitrogen ratio becomes narrow as compared with the fresh material. In soils the ratio of carbon to nitrogen indicates the nature of the organic matter and the extent to which it has decomposed. The ratio of carbon to nitrogen in green plants is 25–40:1, whereas, in field humus it is 10–15:1. Legumes and other materials rich in nitrogen yield a residue in the soil with a narrow carbon-nitrogen ratio. Straw, leaves, and similar substances give a wide ratio. The further the decay has progressed in a soil, the narrower the carbon-nitrogen ratio. Other things being equal, a wide carbon-nitrogen ratio indicates a more fertile soil than one containing a narrow ratio.

Humus.—The plant residues which find their way into soil are rapidly disintegrated by bacteria, and there results a light mass of partially decayed black organic matter—the so-called "humus." This is the storehouse of the soil nitrogen, and as it is decomposed by bacteria the nitrogen is liberated and transformed into nitrates. During the transformation carbon is liberated in relatively greater quantities than is nitrogen, with the result that the carbon-nitrogen ratio becomes narrow and the resulting mass more resistant to bacterial activities. Its composition and value depend upon the material from which it was produced. Proteinaceous materials yield a substance rich in nitrogen. The rapidity with which it is produced also varies with the original material. All of the simple carbohydrates and many proteins are quickly attacked by numerous bacteria, whereas nucleoproteins and cellulose are more resistant and are decomposed by fewer species of micro-organisms. Humus renders the soil light and porous, thus increasing its water-holding capacity and rendering it more easily tilled. However, the greatest effect upon the chemical and biological transformations of the soil has been exerted before the carbonaceous material reaches the stage of humus.

Part of the humus is worked over by soil fungi and incorporated into their bodies, but the greater part of it is slowly oxidized with the formation of a coal-like residue which remains as an inert mass in the soil.

Cellulose.—The woody fiber which constitutes the cell wall of plants is a very resistant carbohydrate, cellulose. Next to water it is the most abundant substance in the vegetable world.

It is to the plant what bone is to the animal. It is especially abundant in the roots, stems, and leaves of mature plants. It constitutes from 30 to 50 per cent of hay and coarse fodder. Straw, husks, and old mature plants contain much larger quantities. Cotton, linen, and paper are examples of nearly pure cellulose. It is insoluble in all the common solvents and is resistant to most chemicals. Cellulose dissolves in ferric chloride, sodium hydroxide, and carbon bisulphide and is broken down by various acids into simpler soluble substances.

Cellulose Decomposing Micro-organisms.—Cellulose finds its way into the soil in large quantities in the residues of plants. Early in the history of the race, man must have observed that cellulose slowly disappears. It was not until after Pasteur announced that cellulose decomposition is a biological process,

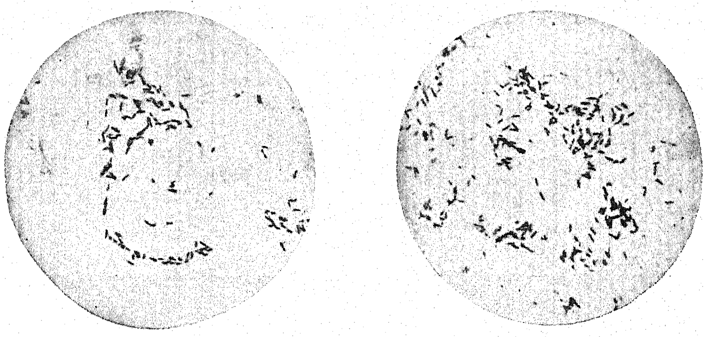


Fig. 53.—Cellulose ferments. (*After McBeth.*)

which he believed due primarily to molds, that it was scientifically investigated. Today various molds, bacteria, and actinomyces are known which decompose cellulose. Physiologically, these may be grouped as anaerobic and aerobic ferments.

Evidence was strong that cellulose decomposes under anaerobic conditions with the production of gases and acids, but nothing definite was known concerning the causative organism until toward the close of the nineteenth century, when a Russian investigator, Omelianski, described two spore-bearing anaerobic organisms which slowly decomposed cellulose. One of these attacked it with the production of hydrogen, the other with the production of methane. They are clostridia resembling the butyric acid group of organisms but do not contain granules that stain blue with iodine, as do the butyric acid ferments. They

produce spores and, together with other anaerobic cellulose decomposing bacteria, occur in peat, marshy soils, river bottoms, manure piles, and other places where there is organic material and absence of free oxygen. In arable soils, cellulose is decomposed in the presence of oxygen due to molds, bacteria, and actinomyces. Kellerman and McBeth isolated very active aerobic cellulose ferments from arable soils. All were rods varying in length from 0.8 to 3.5 μ. Involution forms were observed for only three species. Five species were found to produce spores; twenty-seven were motile. Of these, seven were pseudomonas and twenty-three bacilli. It was shown that a few were facultative anaerobes, but most were true aerobes. The optimum tem-

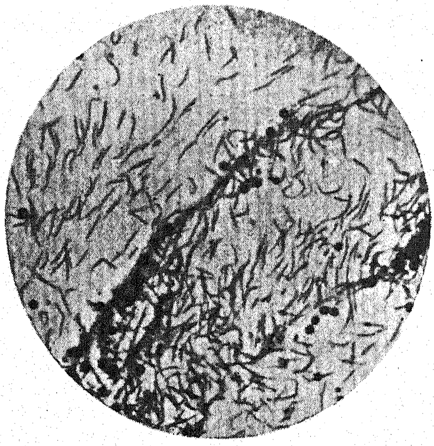


Fig. 54.—Spirochaeta cytophaga. (*After Hutchinson and Clayton.*)

perature lies between 26° and 33° C. They grow rapidly on solid media such as beef agar, gelatin, starch, and potato. Nineteen species liquefy gelatin. They all decompose cellulose and other carbohydrates with the production of acids. Not one produces a gas.

In 1919 Hutchinson and Clayton described a remarkable organism which they isolated from Rothamsted soil and which they named *Spirochaeta cytophaga*. It is a true aerobe. In the active stage it exists for the most part as a thin flexible rod tapering at the extremities. This form passes into a spherical cystlike stage. It appears to be unable to derive energy from any carbohydrate other than cellulose, and curiously enough in pure cul-

tures all the simple carbohydrates appear to be toxic to the organism.

The action of bacteria upon proteins yields amino-acids. These upon further decomposition yield ammonia and nitrogen-free compounds. Among these nitrogen-free products are carbolic acid and related compounds. These substances in time, if permitted to accumulate in the soil, would become injurious to plants including the microflora of the soil. Recent work at Rothamsted has shown that numerous soil micro-organisms possess the remarkable power of utilizing these compounds as their sole source of energy.

Function of the Cellulose Ferments.—The cellulose ferments are especially valuable in liberating the carbon which finds its way into sewage in the form of cellulose. In the septic tanks there are millions of these micro-organisms, busily changing this resistant organic material into gaseous products, and today many large cities depend upon them for the disposal of their waste. They also play a great part in the purification of a city's water supply. Omelianski considers them of prime importance in the formation of soil humus.

The cellulose ferments break the plant residues into less complex organic compounds which are decomposed by other micro-organisms with the generation of large quantities of organic acids. These acids react with the minerals of the soil and render them available to higher plants. This very likely explains the beneficial results obtained when raw rock phosphate and stable manure are used on phosphorus-poor soil. The fermentation of the cellulose yields acids which render soluble the phosphorus. However, the production of acids may at times become excessive, thus giving rise to the sour humus of moors and heaths.

It is well known that the fermentation processes in the soil resulting in the decomposition of organic matter may give rise to large quantities of carbon dioxide, methane, and hydrogen. The hydrogen and methane do not all pass into the atmosphere, but, according to the researches of recent investigators, furnish energy to numerous other soil micro-organisms. The importance of this, however, still remains almost wholly for future workers to develop.

The cellulose ferments also perform direct beneficial functions in the soil, for instance in the liberating of plant food which is bound up in the residues of plants. Heinze has attributed to bacterial activities much of the benefits resulting from summer

fallowing. He found in quantitative studies that bacteria were more numerous in fallow soil than in cropped soil. He thinks the benefit comes from their activities in rendering the cellulose more suitable as a carbon supply for the Azotobacter, thus causing the increase of soil nitrogen in fallow land noted by a number of recent workers. Hence, they are the organisms which decompose the cellulose of straw so it can be utilized by the Azotobacter in the fixation of atmospheric nitrogen. More recent investigators have shown that under suitable conditions the Azotobacter may fix 325 mg. of nitrogen for every 100 Gm. of straw decomposed. At this rate there would be a gain of 7 pounds of nitrogen for every ton of straw utilized. This, in addition to other beneficial effects, the farmer loses when he burns his straw. The cellulose ferments are being used in the synthetic production of manure. Investigators at Rothamsted have shown that if a straw heap is treated with the correct proportion of ammonia and inoculated with cultures of *Spirochaeta cytophaga* it changes into a substance having the appearance of a well-rotted manure. Field tests of the product show that it produces an effect very similar to that of natural barnyard manure. Recently an attempt has been made to use cellulose ferments in the production of acetic acid. A mash of cellulose material (such as sulphide pulp or straw) is inoculated with fermenting vegetable material such as stable manure and maintained under aerobic conditions at a temperature between 25° and 60° C. The cellulose undergoes acetic fermentation and calcium carbonate, or some other suitable substance, is added to neutralize the acid as it is produced. Later the acid is separated from the fermenting mass and purified. Some day probably this process will be improved upon and very active pure cultures used.

CHAPTER XVIII

THE NITROGEN CYCLE

It is carbon that stamps a compound as organic, but it is nitrogen that determines the extent of life on this planet. The whole atmospheric ocean of carbon dioxide is available to growing plants, but only the small quantity of nitrogenous compounds within feeding area of their roots is available. Carbon dioxide is utilized by all green plants, free nitrogen by a few and then only in the presence of micro-organisms. It is thus seen that bacteria play a greater part in the nitrogen cycle. This cycle has been given much more consideration by investigators than the cycle of the other elements.

Occurrence of Nitrogen.—Elementary nitrogen constitutes 79 per cent of the atmosphere. It is contained in the mineral matter of the earth only in minute quantities, but enters into the structural material of all plants and animals. Consequently, it reaches the soil as a part of their bodies, and becomes incorporated into its surface. This is the source of nitrogen for growing plants.

"The proportion of nitrogen to carbon in the human body is 1:2, in the atmosphere it is 5500:1. A human adult contains in his body about 12 pounds of nitrogen and approximately 25 pounds of carbon. Over every square foot of the earth's surface rises a column containing some 1500 pounds of nitrogen, and only ¼ pound of carbon. The demand and supply of these two elements appear therefore at first sight, to be altogether out of all proportion favorable to nitrogen. Yet, in point of fact, the practical problem of securing an adequate supply for the sustenance and expansion of life is incomparably more complex in the case of nitrogen than in the case of carbon. The reason for this somewhat remarkable inversion is to be seen in the fact that nitrogen is readily accessible as food for living organisms only when it occurs in certain chemical combinations, and nitrogen thus combined is far from plentiful."

Properties of Nitrogen.—Pure elementary nitrogen is a colorless, tasteless, odorless gas. It is distinguished primarily by nega-

tive properties. It will not support combustion, nor will it support animal life. It is not poisonous, just inactive. It combines directly with only a few of the other elements, but can be indirectly made to combine with many and in this way yield numerous interesting compounds possessed of remarkable characteristic properties. It is incombustible under ordinary conditions, but under the influence of a powerful electric current it can be made to combine with oxygen. The combustion, even under this condition, does not extend to the surrounding air, for heat is absorbed and must be supplied from some external source.

A world whose atmosphere consisted solely of nitrogen could not support life even for a few minutes. "The dead listless air would choke out their lives almost immediately not because nitrogen is in any way poisonous in the sense that coal gas is, but merely because it is so chemically indifferent that it cannot support the combustion required for our lives. Men and animals require oxygen and are indifferent to nitrogen.

"Perhaps, however, the most extraordinary thing of all on such a planet would be the incombustibility of everything which burns freely in the atmosphere of our earth. No matter how hard we tried, no candle nor oil lamp could be lighted, and paraffin oil could be poured upon white hot coal without catching fire! Indeed, the oil would only serve to quench the heat of the coal just as water would! Our cheerful coal fires would be an impossibility on such a planet, for coal would be as incombustible as gold or stone. In fact, it would be a perfectly useless mineral, and not, as on our earth, the source of untold wealth and power. It is true that by its destructive distillation gas could be obtained, but as this would be as incombustible as nitrogen itself, it too would be quite useless."

Although in the free form nitrogen is inert, in the combined form it is very active. The compounds of nitrogen are extraordinary. The most beautiful dyes contain it. It is tucked away in our most powerful explosives. It enters into the composition of our most delicate perfumes, and it is found in the vilest smelling compounds. It plays its part in our most useful drugs, it is contained in the most powerful poisons; and, most wonderful of all, it is the basis of living protoplasm.

Proteins.—Most of the nitrogen-carrying compounds composing the bodies of animals and plants, including bacteria, are proteins. They are also the principal nitrogenous compounds decomposed by micro-organisms. For these reasons it is requisite

that the student have at least a nodding acquaintance with a few of the fundamentals of protein chemistry.

Proteins are complex organic compounds composed of the elements carbon, hydrogen, oxygen, nitrogen, and usually sulphur. In addition to these some proteins contain phosphorus, iron, copper, iodine, and even other elements. The quantity of the various elements in the different proteins varies between comparatively narrow limits, as may be seen from the following:

Elements	Per cent
C	50.6 to 54.5
H	6.5 to 7.3
N	15.0 to 17.6
O	21.5 to 23.5
S	0.3 to 2.2
P (when present)	0.4 to 0.9

Viewed from the chemical standpoint a protein has a huge molecule, complex in structure, unstable in character, and hence prone to chemical change. So large and intricate is the protein molecule that chemists for generations have been baffled in their attempts to gain an adequate conception of its nature. Some conception may be gained of the size of the molecule from its calculated molecular weight of approximately 16,000 and its complexity from the formula $C_{239}H_{389}O_{78}N_{58}S_2$, which has been suggested for one of the proteins in white of egg. Many of the proteins are soluble in water, salt solutions, or alcohol in which they occur as colloids, in which condition they do not pass through parchment paper.

Numerous natural proteins occur in both plants and animals. These are divided into groups, depending upon the complexity of their structure and their solubility in various solvents—pure water, saline solutions, alcohol, and their like. They are decomposed by means of acids, alkalis, superheated steam, and the enzymes secreted by plants and animals, and yield some nineteen or twenty compounds known as amino-acids. In the protein molecule the amino-acids are linked together to produce the mosaic of the various proteins somewhat as the twenty-six letters of the alphabet are grouped giving various words. The so-called "simple proteins" on digestion yield only amino-acids and their derivatives, whereas the complex proteins yield not only amino-acids but also other compounds. Micro-organisms build up and break down both simple and complex proteins. It is the activ-

ities of the microbes which render the nitrogen of the proteins available to higher plants. Bacteria are, therefore, a vital link in the nitrogen cycle.

Outline of Nitrogen Cycle.—Nitrogen occurs in plants and animals primarily in the form of protein. The plant protein may be eaten by the animal, and there results animal protein. Either may reach the soil and decay. The nitrogen eaten by animals may be deposited as tissues of the animal or excreted as urea, hippuric, or uric acid. These products are decomposed by microorganisms with the formation of ammonia.

Either the plant or animal proteins may reach the soil where they decay with the formation of the simpler compounds—

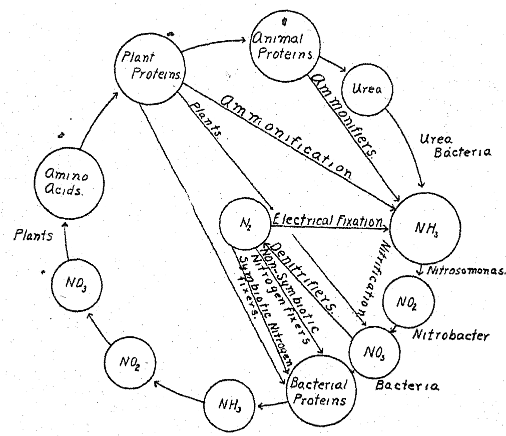


Fig. 55.—Illustrating nitrogen cycles in nature.

albumoses, proteoses, peptones, peptids, and amino-acids. The amino-acids are then deaminized with the production of an acid and ammonia. The process is spoken of as ammonification.

The ammonia does not accumulate in the soil but is acted upon by other bacteria, the nitrosomonas, with the formation of nitrous acid. This is quickly taken up by the nitrobacter and oxidized to nitric acid which reacts with bases in the soil with the formation of nitrates. Plants use nitrates as their main source of nitrogen and build from them and carbon dioxide amino-acids, peptids, peptones, proteoses, albumoses, and finally plant proteins—and the nitrogen has completed its cycle.

At different stages in this cycle chemical or biological agencies may liberate elementary nitrogen. It is then inert and useless to

animals and most plants and can be made to reenter the nitrogen cycle again only by the addition of energy.

Portals of Entry into Cycle.—Nitrogen appears to have been the leftover after the completion of the earth, and as such occurs primarily in the inert atmospheric form. Thus, we ask: How did it enter the cycle, and when once removed, how can it again return? Each flash of lightning in the billions of storms which have occurred since the founding of the world has caused it to combine. Every volcanic eruption has thrown it into the atmosphere to be washed into the soil by the succeeding storm. The magnitude of this may be seen from calculations made by Arrhenius. He finds that the quantity brought to the earth's surface each year amounts to the stupendous quantity of 1500 million tons. This is scattered with indifference on water, stones, and soil; and the quantity reaching each acre is small, and its concentration by man is an impossibility. Such a task would be somewhat similar to the extraction of gold from sea water. It has been estimated that if all the gold in sea water were extracted and apportioned evenly to all the inhabitants of the globe we would each be millionaires three times over. But it would cost more to recover it than the value of the gold.

Some years ago Hellriegel and Welfarth discovered that legumes when associated with certain bacteria possess the power of using atmospheric nitrogen. This is built into proteins and on decay becomes available to all plants. Still later Beijerinck discovered large yeastlike micro-organisms which live free in the soil and take from the atmosphere nitrogen and build it into complex compounds. Every year since this date the number of organisms found to possess this valuable property has been added to, until today we know that in a measure it is possessed by most micro-organisms. Very recently Lipman has shown that even some nonlegumes may to a limited extent assimilate atmospheric nitrogen. True, each plant or micro-organism fixes but a minute quantity and the quantity gathered on an acre yearly is not large, but given time and nature's process of concentrating, it becomes considerable, as is exemplified by the deposits in South America and the excessive concentration found in some western soils.

Within recent years the chemist has learned to substitute the electric arc or the electric current for lightning in order to capture atmospheric nitrogen. In the Birkeland-Eyde process air passed through an electric arc is fanned into a broad disk by a

magnetic field, and from this there issue oxides of nitrogen. In the cyanamide process a strong electric current is passed through a mixture of lime and coke. The result is calcium carbide. When a stream of nitrogen is passed over this white hot compound lime nitrogen results. This can be used directly, or changed to ammonia by treating with superheated steam. Today in countries where the required energy can be obtained from either natural or artificial waterfalls large quantities of nitrogen are being fixed and are successfully competing with the

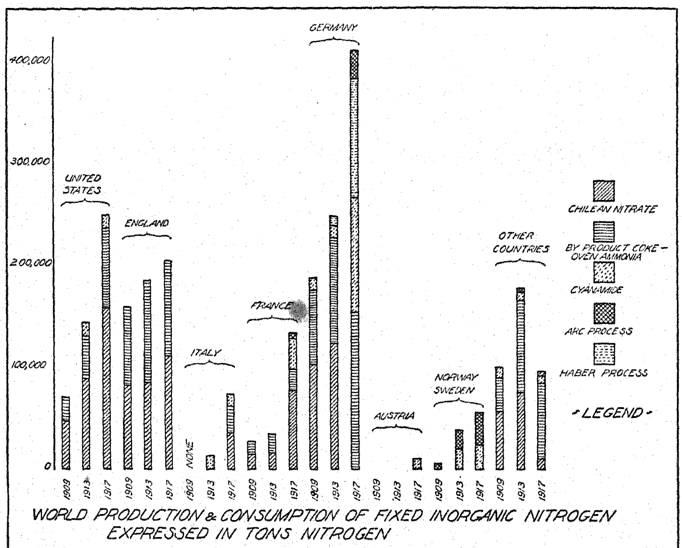


Fig. 56.—Illustrating production and consumption of fixed inorganic nitrogen expressed in tons.

natural product. Where water power is not available, nitrogen is also being captured but by a different process. A mixture of hydrogen and nitrogen is passed over uranium, osmium, or platinum. These act as catalysts causing the hydrogen and nitrogen to combine directly with the production of ammonia.

Leaks in the Nitrogen Cycle.—There are few gates through which nitrogen may enter the cycle but many by which it may leave. It is liberated when the bodies of plants and animals are burned. Millions of pounds of it reach the sewers, and from

here are carried to rivers, lakes, and oceans. The compounds containing it are broken down, and the nitrogen is transformed into ammonia, nitrites, or nitrates. Various micro-organisms liberate from these gaseous nitrogen. Each discharge of a fire-arm liberates it. Modern explosives are returning each year millions of pounds to the atmosphere. In some of the battles of the World War enough nitrogen was liberated to produce the food of the civilized world for a year. Its energy is being used in the digging of our canals, in the making of our tunnels, in the construction of our railroads, and in the mining of our coal. In each case it leaves the cycle only to return when outside energy is supplied.

Ammonification.—The soil is a large digestive system in which plant and animal residues are worked over so they may serve again as food for higher plants. The digestion is due to micro-organisms which decompose the organic compounds so that they may obtain energy and building material. Incidentally, some of these decomposition products serve as food for higher plants. This is especially true of the nitrogen-carrying compounds. Micro-organisms split proteins, and proteoses result. Proteoses are decomposed with the production of peptones; peptones yield amino-acids. These in turn are oxidized or hydrolyzed, forming ammonia, and nitrogen-free compounds. These latter products are used by the micro-organisms as a source of energy. Small quantities of the nitrogen have been used in the building of their body proteins, but by far the greater quantities of it are left in the soil to be changed into nitrites and finally nitrates by other micro-organisms. Hence, we speak of the decomposition of nitrogenous substances by biological agencies with the production of ammonia, as ammonification.

Materials Ammonified.—Although all nitrogenous substances can be decomposed by micro-organisms and usually with the formation of ammonia, the speed with which this transformation occurs varies with the compound. Urea and the simple proteins (especially those found in fish and blood) are rapidly decomposed by many bacteria. The complex proteins found in cow manure and cotton seed meal are more slowly decomposed, whereas those in leather and bone are quite resistant. Some fungi (but not bacteria) are known to be able to decompose the commercial nitrogenous fertilizer, cyanamide, with the production of urea. This latter product is readily transformed into ammonia by various bacteria.

The speed with which compounds are decomposed varies greatly, but ultimately all organic nitrogen is ammonified. Even such resistant or poisonous substances as chitin, quinine, strychnine, morphine, nicotine, and even the toxins produced by pathogenic bacteria are no exceptions.

The nitrogen of straw is slowly ammonified. This is due to the nature of the nitrogen compounds occurring in the straw and also to the carbohydrates present, thus, making it unnecessary for the micro-organisms to split the proteinaceous products. The general rule seems to be as follows: The addition of a carbohydrate to a medium delays the decomposition of the nitrogenous materials. Hence, when carbohydrates are added to a soil rich in straw or other plant residues they reduce for the time being the speed with which ammonia is produced. This may actually manifest itself by a decrease in the crop yield. It might thus be said that the speed with which ammonia is produced in a soil varies with the quantity and kind of manure applied. It also varies with the soil, for a rich sandy soil supplied with optimum moisture may ammonify many times faster than a depleted soil, or even a rich, tight, clay soil.

The optimum moisture content (if stated as percentage of water in a soil) varies greatly in different soils. For a tight clay it is very high and for a sand very low. However, if the water content of the soil is expressed in terms of its water-holding capacity it has been found that ammonification is at its maximum when the soil contains 60 per cent of its total water-holding capacity. The ammonia produced in a soil increases as the water content increases until this limit is reached. Above this there is a sharp drop in the ammonia produced. The reason for this can be readily understood when it is recalled that in the last stage of ammonia production oxygen enters and ammonia is split off, consequently, aeration increases ammonification. It is rapid in fallow and hoed soils in which potatoes, corn, and beets are planted but it is slow in oats, wheat, and alfalfa soil.

The ammonifying organisms grow best in a neutral or slightly alkaline medium. Acid soils ammonify slowly, and probably the small quantity produced is due primarily to molds. Either lime or limestone added to such a soil accelerates the process.

Micro-organisms decompose nitrogenous substances primarily to obtain energy for the carrying on of their life processes. They must also have certain elements to be used in building their

bodies. This is especially true of phosphorus which is needed in large quantities. Therefore, a soil having a goodly supply of this element in an available form liberates larger quantities of ammonia than a soil poor in phosphorus, provided other conditions are ideal.

Organisms Causing Ammonification.—Although it had been known for a long time that small quantities of ammonia occur in all arable soils, its formation was not known to be due to bacteria until 1893, when Müntz and Condon demonstrated that ammonia is not produced in sterile soils. Following this, they and other workers tested many bacteria, yeasts, and molds and found that numerous micro-organisms possessed the power of producing ammonia. Even many of the pathogens when grown under ap-

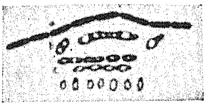


Fig. 57.—B. mycoides. (*After Conn.*)

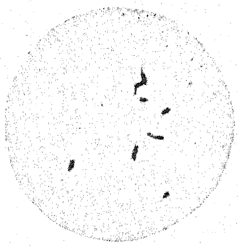


Fig. 58.—Ps. fluorescens. (*After de Rossi.*)

propriate conditions produce ammonia. *B. mycoides* and related spore-bearing organisms were found to be very active ammonifiers under laboratory conditions; hence, it was quite generally accepted that they played a very prominent part in the production of ammonia in soil. However, the work of Conn makes this appear very doubtful, for he found that these organisms, while in the soil, exist primarily as spores, and are thus inactive. But, he did find that *Ps. fluorescens* and related nonspore-forming organisms take part in the production of ammonia in manure and manured soils.

Numerous soil fungi have been studied which rapidly produce ammonia, and it is the opinion of many soil workers at the present time that these play a more prominent part in ammonification in soil than do bacteria. Probably there is a variation in different soils, depending upon the proteinaceous substance present. One organism may act best on one substance and in one

soil, whereas another organism may be at its maximum efficiency in an entirely different soil working on other material. As these organisms exist in soil probably there is a division of labor. Some rapidly split the complex proteins; others act on the albumoses; and still others act on the amino-acids. There are bacteria which quickly break down urea with the production of ammonia. This may often be detected by its odor in a stable on a warm day. Although we find a great variation in activity and substance on which the ammonifiers act, yet they are all working in keeping with the general scheme of things—the working over of old discarded material and rendering it suitable again to be used by plants and eventually by animals. The numbers in a soil vary, but probably from 10 to 25 per cent of the soil microflora are active ammonifiers.

Importance of Process.—We have seen that ammonification is a process by which complex compounds are changed into simple ones. It is obvious that higher plants cannot feed on fish, meat, or manure, but when these are changed into nitrates they become excellent sources of plant food. The process of ammonification then is one step in the production of available plant food. Therefore, anything which will accelerate it during the growth of the plant is beneficial, whereas anything which retards it is detrimental.

Moreover, anything which would accelerate it during the wet seasons when the plants are not in the soil is injurious, because as the nitrogen changes into ammonia (and especially when transformed into nitrates) it is soluble and can be leached from the soil. The quantity of ammonia in a good arable soil is about 4 pounds per acre-foot. Occasionally ten times this amount may be found in pasture or heavily manured soil. In special cases even larger quantities may occur, but then abnormal plant growth occurs.

Nitrification.—Ammonia does not accumulate in the soil, but as rapidly as it is produced it is transformed into nitrites and then into nitrates—a process known as nitrification.

Pure cultures of the nitrifying bacteria were first obtained by the Russian investigator, Winogradsky, in 1890. He showed that these organisms are sensitive to organic matter and, hence, did not develop on the gelatin plates which were used at the time in the study of soil organisms. He found the following quantities of organic matter to inhibit the growth and action of the nitrifying organisms in alkaline solutions:

	Peptone, per cent	Asparagine, per cent	Urea, per cent	Glucose, per cent	Ammonia, per cent
Nitrosomonas....	0.2	0.3	?	0.2	
Nitrobacter......	1.25	0.5 to 1.0	1	0.2 to 1.03	0.015

He grew them for some time in media containing only mineral salts, after which they were transferred to gelatin plates. A few colonies developed, but having learned that none of these were the organisms sought, he concluded that they might be at the points where inoculations had been made but no growth was visible. He then inoculated media with material picked from these points and in this way obtained the organisms in pure cultures. Successful platings were made only after Kühn introduced silica jelly for such purposes. Later it was shown by Beijerinck that agar can also be used after it is carefully washed free from all soluble material. Winogradsky found the nitrite producers to grow either as small oval, motile rods with polar

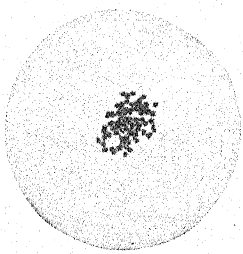


Fig. 59.—Nitrifying bacteria, nitrococcus. (*After Bonazzi.*)

flagella, or in larger nonmotile globular forms. He named them *nitrosomonas* and *nitrococcus,* respectively. Both possess the power of transforming ammonia into nitrites. The organisms which oxidize the nitrites to nitrates he designated *nitrobacter*. These are small nonmotile rods and transform the nitrites into nitrates as fast as they are produced in the soil, and it is only under abnormal conditions that either ammonia or nitrites accumulate.

In fact the speed of the reaction is governed by the rapidity with which ammonia is produced in the soil. Claims have been made that there are individual organisms in the soil that can bring about the two processes. Recently Sack reported that he

had isolated from the soils of Holland a nonmotile, gram-negative organism, *Nitrosomonas groningensis* capable of oxidizing ammonium salts to nitrites and to nitrates. He found that the organism thrives best in the presence of a large amount of organic matter; that it attacks cellulose and liquefies gelatin; and that, in the absence of oxygen, it reduces nitrates to nitrites and forms ammonia from proteins. If later work confirms these findings, it will revolutionize our ideas concerning the specialized activity of nitrifying organisms.

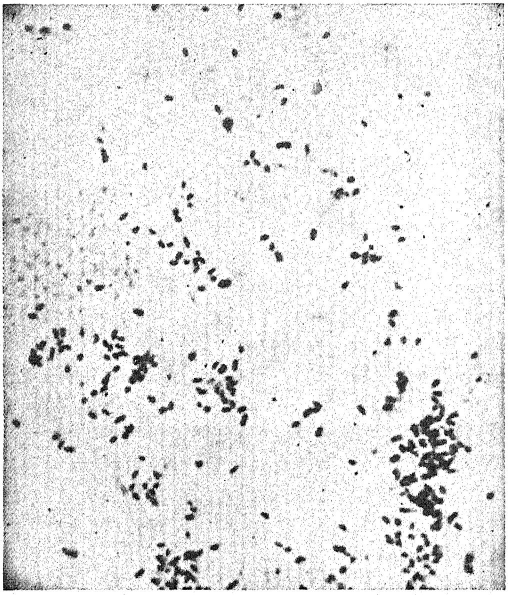


Fig. 60.—Nitrifying bacteria, nitrobacter. (*After Gibbs.*)

Distribution of Nitrifiers.—Probably the nitrifying bacteria were some of the first living organisms on this earth. Even today they are the pioneers who prepare the soil for other plant life. Müntz has found them on the rocks of Alpine summits where no other life exists. The organisms obtain their carbon from the atmosphere and their energy from the nitrogen compounds brought to them by the snow and rain. The ammonia is converted into nitric acid. This together with the carbon dioxide of the air, renders available the essential elements contained within the rocks.

They have been found in all normal cultivated soils and even in the sands of the desert. In humid regions they are confined principally to 18 inches of the surface, but in districts where the soils are well aerated they have been found at a depth of 5 to 6 feet. They are not usually found in the air or in rain water, but river water and sewage contain them, and they are usually present in well waters. In the case of deep wells their origin is probably due to surface drainage which has found its way into the well, as the water of deep wells is not their natural habitat.

Factors Governing Nitrification.—Winogradsky concluded from early work that all soluble organic substances must be decomposed before nitrification can take place, and all ammonia must first be converted into nitrites before nitrobacter can commence work. It is self-evident that this cannot be rigidly applied to soil, as soils which have received large quantities of barnyard manure rapidly nitrify. Moreover, it has been shown that the soil humus is not detrimental but usually very favorable to the nitrifying organisms in soil. These apparent differences come from the fact that laboratory tests were made in solutions, the aeration of which was poor. In the soil it is good. Furthermore, the quantity of soluble organic material in soil is usually below that which Winogradsky found to be injurious. Ammonia exists in the combination of salts which are not injurious to the nitrifiers. In peaty soil and even in other soils where there is an acid reaction or a strongly alkaline reaction nitrification does not occur but can usually be started by neutralizing the acid or removing the excessive quantity of soluble salts.

The nitrifying bacteria, although light-avoiding are heat-loving, as illustrated by the following results: King found that there was produced during a year in an acre-foot of soil 120 pounds of nitrates at 1° C.; 150 pounds at 9° C.; 329 pounds at 20° C.; and at 35° C. there was produced 747 pounds.

They are also dependent upon a suitable moisture content of the medium in which they are growing. When only small quantities of water are present in a soil nitrification is slow. If too great an amount is present the nitrates disappear. The percentage quantity necessarily varies with different soils. However, the senior author has shown that if the water-holding capacity of the soil is determined and 60 per cent of this quantity be added to the soil, nitrification is at its maximum insofar as water is concerned.

The nitrifying bacteria are similar to other plants in that they can live on inorganic salts. Even carbon dioxide suffices as their source of carbon. The green plants with few exceptions are the only living organisms known to use carbon dioxide. These get the energy necessary to do this through the action of their chlorophyll on sunlight. The fuel which the nitrifying ferments burn in their tiny engines is ammonia in the case of nitrosomonas and nitrites in the case of the nitrobacter. The ashes from this quiet but interesting combustion are nitrates. Workers have traced a definite relationship between the amount of ammonia oxidized and the carbon assimilated, as may be seen from the following:

	Experiment			
	1	2	3	4
	Mgm.	Mgm.	Mgm.	Mgm.
Ammonia oxidized (expressed as nitrogen)	722.0	506.1	928.3	815.4
Carbon assimilated	19.7	15.2	26.4	22.4
Ratio—nitrogen: carbon	36.6	33.3	35.2	36.4

Hence, 30 or 40 pounds of nitrogen are oxidized in soil by these organisms for every pound of carbon they assimilate.

Function of the Nitrifiers.—The nitrogen used by most plants is in the form of nitrates; so the main function of the nitrifiers is to render this element available to the higher plants. The acids produced have an indirect effect in rendering available phosphorus and potassium. However, the soluble nitrates are readily washed from the soil, and in regions where the annual rainfall is large or when excessive quantities of irrigation water are applied to a soil the loss in the drain water may even exceed that taken up by the growing plant. This, the careful farmer tries to prevent by growing crops on his soil while nitrification is rapid.

Nitrate Transformation.—Under normal conditions nitrates do not accumulate in the soil but are removed nearly as fast as produced. This removal takes place in the following manner:

(1) They are taken up by the growing plants and transformed into proteinaceous material. This is a legitimate loss, and, if conditions could be made ideal, would be the main way by which they disappear. It is evident that its magnitude depends upon the size and kind of crops grown. (2) Large or small quantities of nitrates may be washed from the soil, depending upon the nature of the soil, the plants growing upon it, and the volume of water which reaches it. In some deep arid soils this may be zero, whereas in shallow well-drained humid or heavily irrigated soil the quantity carried from the soil by this method may exceed that taken up by the crop. It is a dead loss and every means should be taken to prevent it. (3) The nitrates may be assimilated by micro-organisms and built into bacterial proteins. Later, these may be nitrified and again rendered available to higher plants. This process is most rapid in soils containing decaying organic material and under some conditions may become great enough so that the micro-organisms compete with the higher plants for the limited supply of nitrates in the soil. This is exemplified when straw or fresh manure is plowed under a few days or weeks before a new crop is planted. Under these conditions the straw or manure may have a distinct retarding effect upon the crop, because the carbon compounds will permit the soil microflora to assimilate the soluble nitrogen compounds previously formed in the soil. For a similar reason manures containing large quantities of organic material have a more lasting influence upon the productivity of the soil than do commercial fertilizers. (4) The nitrates may be partially reduced with the production of nitrites or even ammonia. Many organisms exist in the soil which have the power partially or completely to bring about these reactions. The reduction of nitrates to nitrites may occur under aerobic conditions, but the transformation of the nitrites to ammonia is confined almost entirely to anaerobic condition. Thus, it occurs only in peaty, swampy or water-logged soil. Neither process is of any economic importance in the soil as the nitrites or ammonia produced are reoxidized to nitrates as soon as aerobic conditions are established. The process is at times made use of in cheesemaking. Nitrates are added to the curd and are decomposed by the bacteria present, thus preventing them from decomposing the lactose resulting in the formation of hydrogen and carbon dioxide which may cause ill-formed cheese. (5) The nitrates may be completely reduced, the nitrogen escaping as free nitrogen. This process is

known as denitrification, and when it occurs in soil is of economic importance, as the nitrates are permanently lost.

A rather large number of denitrifying bacteria have been described and often named *Bacterium* or *Bacillus denitrificans* with Roman numerals to designate the specific variety. *Achromobacter stutzeri* is a common variety, and *Pseudomonas fluorescens, Bact. pyocyaneum* and *Bact. hartlebii* all possess the power. However, the reduction occurs only under appropriate conditions, such as in the presence of nitrates; and suitable organic material; and in the absence of free oxygen.

Factors Influencing Denitrification.—Denitrification can take place in a soil only when a source of carbon is present, and the form in which the carbon exists is significant. Denitrifiers need large quantities of organic food and develop best in the presence of the fresh plant and animal débris. From the standpoint of production it would thus be unwise for the farmer to use heavy dressings of nitrate and fresh manure, or to plow under with the nitrates green-manuring crops. Partly decayed barnyard or green manures do not appear to favor denitrification.

In very wet soils oxygen is excluded. Here the bacteria turn to the soil nitrates for oxygen and liberate the nitrogen. In well-aerated soils this does not occur as the denitrifiers appear to be only facultative anaerobes. Denitrifiers are mostly heat-loving microbes, and hence have an optimum temperature relationship considerably higher than that which occurs in the average soil.

Functions of Denitrifiers.—When acting in the farmer's soil, the denitrifier is a robber bacterium lying in wait until the banquet has been prepared by the honest-toiling nitrifiers. Then the denitrifier slips in and wastes the precious plant food. But from what has been said concerning its habits, we find this microbe a robber by force of circumstance imposed upon him by the farmer's injudicious handling of his soil. However the denitrifier has a specific function in returning to the atmosphere the huge quantities of nitrogen which find their way in the form of organic compounds into the septic tanks of large cities. Moreover, thousands of tons of organic nitrogen are carried by streams to lakes and oceans. The nitrogen of this must be returned to the atmosphere so as to start again on its wonderful journey of construction, or in the case of war, destruction. How shall this be accomplished other than through the action of the denitrifiers?

CHAPTER XIX

NITROGEN FIXATION, NONSYMBIOTIC

Plants require at least ten elements for their normal growth and fruition. Three of these (nitrogen, phosphorus and potassium) are in soil only in small amounts as compared with the quantity required by the growing plants. One, nitrogen, is the limiting factor of crop production in most soils. The ancients, without a knowledge of these fundamental principles, supplied these essential elements to the soil in the form of manures, animal refuse, bones, and fish. Even the Indians taught the early settlers of this country to drop a fish into each hill of corn they planted. And ever since the discovery of the nitrate beds of South America this product has been used on the soils of Europe, Asia, and America. Although many new sources of combined nitrogen had been discovered and utilized, yet William Crookes, the discoverer of the Crookes tube, startled the world in 1898 by declaring that it was nearing the limit of wheat production and that by 1931 the bread eaters would have to turn to other grains or restrict their population, whereas the rice and millet eaters of Asia would continue to increase. He pointed out that the quantity of available combined nitrogen was going to be a limiting factor unless means be obtained for the utilizing of the atmospheric nitrogen.

From that date on many careful students have sounded the warning that the population is rapidly reaching the saturation point and must be met by a restriction of the population or an increased production of foodstuffs. New arable land is fast diminishing, and in the very near future more food can be produced only by the removal of the limiting factor of production. This at the present time, on the majority of soils, is the available nitrogen. Nitrogen fixation has consequently become one of the most vital problems which confront not only the tiller of the soil but mankind in general. This general conclusion has been reached by all who have given the subject careful thought, and as a consequence nitrogen fixation processes are and have been for a number of years the subject of intensive investigations and development in many countries. This is especially true in

Germany, France, Italy, England, Norway, Japan and the United States.

Nitrogen fixation on a commercial scale was first accomplished scarcely a quarter of a century ago yet in 1928 53 per cent of the 1,822,600 tons produced in the world was from air-fixation processes. During the World War the Germans developed the synthetic processes to such a degree that they became independent of the natural occurring supply. This promises to be the case also with other nations in the near future. At present the requisite nitrogen can be fixed both for the arts of peace and war. However, such nitrogen is expensive, and it will be some time before it can compete with nature's method of fixing it in each acre of agricultural soil. It is essential, therefore, to learn the methods and conditions under which the microbe fixes nitrogen for man.

Isolation of Nitrogen Fixers.—During the last quarter of the nineteenth century Berthelot demonstrated that there existed within the soil chlorophyll-free micro-organisms capable of fixing atmospheric nitrogen, and that they function best at summer temperatures, between 50° and 104° F. Heating the soil to 230° F. was found to stop the process. He further showed that these organisms are aerobic and function best in soil with a moisture content of from 12 to 15 per cent. Carbon, hydrogen, and small initial combined nitrogen content were found to be essential. The fixed nitrogen was insoluble in water; hence, he concluded that it was proteinaceous. He considered that the quantity fixed by micro-organisms was limited, as the ones studied by him drew from the atmosphere only so long as the quantity in the medium was not great.

Berthelot's results were published in 1893, and a few weeks later additional data were furnished by the Russian bacteriologist, Winogradsky. He employed a nutrient medium free from combined nitrogen but containing dextrose and mineral salts. Fifteen separate species of soil bacteria were isolated; only one, a long spore-bearing bacillus, grew well in nitrogen-free media and fixed appreciable quantities of nitrogen. The organism he christened *Clostridium pasteurianum*. Later Bredemann showed this organism to be a strain of the common anaerobic butyric acid bacilli (*Clostridium butyricum*) which can be isolated from all soil and under suitable conditions always fixes some nitrogen. Winogradsky found the organism fixed 2 mg. of nitrogen per gram of sugar, while later tests have shown gains up to 6 mg. per gram of sugar decomposed.

A German investigator, Caron, next used in vegetative experiments pure cultures of the bacteria most frequently encountered in natural soils. Some soils received bouillon cultures, whereas others received only sterile bouillon. The crop yields were usually in favor of the inoculated soil, but wide variations occurred from season to season. Exceptionally good results were obtained with a spore-bearing bacillus which he termed *Bacillus ellenbachensis*.

This work led to the commercial exploitation of his cultures, one of which, "alinit," was the subject of much study and discussion. Later work showed alinit to contain two closely related

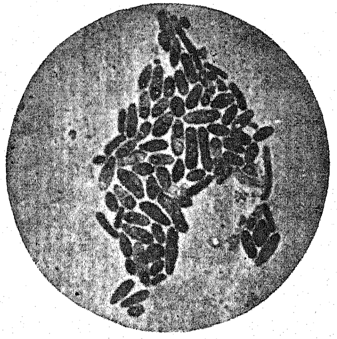


Fig. 61.—Clostridium pasteurianum. (*After Winogradsky.*)

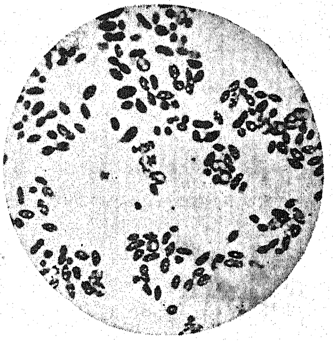


Fig. 62.—Azotobacter chroococcum. (*After Löhnis.*)

bacilli which were designated *B. ellenbachensis* A and B. Both possessed the power of fixing nitrogen to a limited extent. However, later tests with "alinit" have failed to a great extent to confirm the claims of its exploiters.

In 1901 the Dutch bacteriologist, Beijerinck, described a large yeastlike aerobic organism which he named *Azotobacter chroococcum*. These organisms in routine laboratory experiments frequently fix 10 to 15 mg. of nitrogen for each gram of sugar assimilated, and under more favorable conditions even much higher gains have been reported. Since that time many bacteria, yeast, and molds have been shown to assimilate atmospheric nitrogen under appropriate conditions, and today it would appear that the power of fixing minute quantities of nitrogen is a characteristic of most micro-organisms. Nevertheless, up to the present time the *Azotobacter* group appears to be the champion nitrogen gatherer.

Properties of Azotobacter.—Beijerinck described two species of Azotobacter—*Azotobacter chroococcum,* characterized by the production of chocolate-colored pigment, and *Azotobacter agile,* a rapidly motile organism, producing in solid and liquid media a brilliant green fluorescence. Later a number of strains of these organisms were described. For instance, Lipman isolated from the soil of New Jersey *Azotobacter vinelandii* which Löhnis considers similar to *Azotobacter agile. Azotobacter beijerinckii* usually gives a white growth and in certain stages produces a distinctly yellow pigment. It is a strain of *Azotobacter chroococcum. Azotobacter vitreum,* which shows neither motility nor pigmentation, is considered by Löhnis to be a strain of *Azotobacter agile.*

The organisms appear as short round rods 1 to 2 μ in diameter and 1.5 to 3 μ long. They have a great tendency to change morphologically, the large yeastlike form often changing to small coccoid and rod-shaped types. They also pass over into an amorphous or "symplastic" stage. The organism in this form appears under the microscope either as an unstainable, or as a readily stainable mass without any easily distinguishable organization, and, if not discarded as dead, later gives rise to new vegetative forms.

Occurrence of Azotobacter.—Azotobacter are widely distributed, occurring in most normal soils. Lipman examined forty-six soils from various parts of the world and found *Azotobacter* in about one third. It is generally believed that *Azotobacter* occur commonly in soils which contain sufficient calcium carbonate to effervesce when acid is added and that they scarcely ever occur in acid soils.

Azotobacter are confined almost entirely to the first 3 feet of soil, although they have been found in soil at all depths down to the tenth foot in the very favorably constituted loess soils of Nebraska. They are most active in the upper few inches of soil, as is indicated by the following results obtained by Ashby:

Soil	Depth (cm.)	Average nitrogen fixed (mgm.)
Little Hoos	10	9.23
Little Hoos	20	7.29
Little Hoos	30	4.60

Reports on some Hawaiian soils show them to be equally active at all depths to 4 feet, but this must be considered an exception for the examination of numerous soils in Utah has shown a gradual decrease in nitrogen-fixing powers with depth. The averages of several hundred determinations in both solution and soil media are given below:

Depth of sample	Nitrogen fixed in 100 gm. of soil with 1.5 gm. mannite	Nitrogen fixed in 100 cc. of Ashby's solution with 1.5 gm. mannite
	mgm.	mgm.
First foot........	5.28	2.11
Second foot.......	2.42	0.77
Third foot........	1.55	0.58

Condition for Growth.—The distribution and the physiological efficiency of the nitrogen-fixing organisms, especially of the *Azotobacter* species, are governed by the physical and chemical properties of the soil, foremost among which is its calcium or magnesium carbonate content. Some workers make use of this in obtaining pure cultures, for it is found that by picking out the crystals of the carbonate from the soil and seeding them into nitrogen-free media the likelihood of obtaining the organism is greatly increased. The addition of calcium carbonate to a soil often increases its nitrogen-fixing power, the extent depending on the lime requirements of the soil and on the fineness of the added limestone. It has been suggested that the *Azotobacter* be used as an index of the lime requirements of a soil as they do not tolerate an acidity greater than pH 6.

Food Requirements.—*Azotobacter* probably require for their nutrition the same elements as do the higher plants: Namely, carbon, hydrogen, oxygen, nitrogen, potassium, phosphorus, sulphur, calcium, magnesium, iron, and possibly aluminum, manganese and iodine. These micro-organisms obtain their carbon and hydrogen from organic compounds, preferably from carbohydrates. Oxygen and nitrogen are obtained from the atmosphere, the other elements from the soil.

A marked difference between *Azotobacter* and the higher plants is that the former possess the power of obtaining their nitrogen from the air. In the presence of combined nitrogen, however, they become lazy and obtain their supply from the soil. Small

quantities of nitrogen stimulate the *Azotobacter,* whereas large quantities discourage nitrogen fixation since the organisms live on the nitrates. This may be due to a number of causes: (1) Direct toxic action of the salt; (2) antagonism of other organisms which it favors; (3) the using up of the energy supply by these organisms; and (4) the discouragement of fixation by the use of sodium nitrate. They must also have water and a suitable temperature, conditions that appear to be the same as for the nitrifiers.

From Whence Their Energy?—The nitrogen-fixing organisms differ widely from other plants in their energy requirements. This comes from their causing nitrogen to combine with other elements. Energy must be supplied. This is obtained through the decomposition of organic compounds, particularly one of the carbohydrates.

Normally the energy is obtained from the plant and animal residues brought to the soil and differ in value as may be seen from the following:

Material	Nitrogen fixed in 100 c.c. of solution after 3 weeks
	(mgm.)
Fresh straw	10.0
Fresh stable manure	9.8
Fresh peat	9.3
Green manure	8.0
Beijerinck's mannite solution	5.6

After humidification these materials are even more readily assimilated and nitrogen fixation is greater than when the unhumidified substance is used.

Methods of Fixation.—How different are the ways of man and the microbe! Man fixes nitrogen by means of a gigantic arclight in a chimney through which a current of hot air is blown. The flaming disk has a diameter of 7 feet and reaches a temperature in the neighborhood of 3000° C. The gas which is obtained when dissolved in water yields nitric acid. Or, in another method, air is cooled to —194° C., the nitrogen boiled off, mixed with hydrogen in the proportion of 1 to 3, heated to a temperature of 550° C., and then passed under great pressure over a catalyzer. There results ammonia. This, mixed with oxygen and passed

through platinum gauze, yields nitric acid. Thus, in all synthetic processes great variation in temperature, and complicated, expensive apparatus are used.

When the microbe fixes nitrogen there is also a real conflagration in which plant residues act as the fuel and the bacterial body as the furnace. But how different are the two! The microbe is 90 per cent water. It works in the dark, damp, warm soil, and generates little heat and no light. We know its end-products are not nitric acid, ammonia, or cyanamide but complex proteins. These are similar to those found in plants, and are composed of the same amino-acids. Some are soluble, others insoluble, all are broken down by bacteria and yield food for the higher plants. We do not know the first products produced in their formation nor the marvelous way in which the nitrogen is enticed to join hands with carbon, hydrogen, and oxygen. We can only surmise and hope that the future will unravel the mystery. But when unravelled, as it surely will be, the wonderful advancements of the past decade will seem insignificant beside it.

Soil Inoculation.—High hope was entertained that the nitrogen problem in agriculture had been solved when Caron announced that he had prepared a culture of bacteria which would enable nonleguminous plants to utilize free atmospheric nitrogen, provided certain precautions were observed. Many of the results which he reported from pot experiments were clearly in favor of the inoculated plant. Stoklasa was one of the first to study in detail the commercial preparation "alinit" which was placed on the market as a result of Caron's work. His findings were fully as favorable as Caron's, but the work of others soon demonstrated that alinit, neither in the laboratory nor in the field, had the ability to fix nitrogen. When Beijerinck discovered the free-living aerobic nitrogen fixers, the hope that soil inoculation might be so perfected that it would be beneficial to crops was revived, and since that time many investigators have attempted to inoculate soil to increase its productivity, but usually with negative results. Some have made great claims for soil inoculation. They have found that soils, inoculated with *Azotobacter chroococcum* and adequately supplied with carbohydrates and lime, show an increase in the number of nitrogen-fixing organisms and also an increase in crop yields.

Many workers have noted either no effect or even a detrimental influence when soils are treated with carbohydrates and then inoculated with *Azotobacter*. This may be due to any, or all, of

the following factors: (1) Absence of a suitable environment, such as proper temperature, moisture, aeration, food, and alkalinity; (2) absence of a suitable host from which *Azotobacter* may obtain part of its carbon; and (3) injurious effects due to the decomposition products of the carbohydrate added.

It is evident that soil inoculation in order to be successful must be accompanied by the rendering of the physical and chemical properties of the soil ideal for the growth of the specific organisms to be added. A few organisms placed in a new environment already containing millions of other organisms can never hope to gain the ascendancy over those naturally occurring in the soil, for the latter have been struggling for countless generations to adapt themselves to the environment, and only those which are fitted have survived. The problem becomes even more complicated when we recall that Lipman found the bacterial flora of a soil is in many cases entirely changed by climatic conditions. It would appear that to ever make soil inoculation a success the chemical, physical, and even the biological condition must be made suitable for the growth of the specific organism added. Furthermore, strains of the organisms must be used which have been evolved under similar climatic conditions.

Soil Gains in Nitrogen.—It is well established that many micro-organisms, either when grown alone or in combination with other micro-organisms, possess the power of fixing nitrogen. Many of these have been obtained in pure culture and their morphology and physiology carefully studied. The most favorable conditions for the maximum nitrogen fixation of pure cultures in liquid solutions have been accurately determined. Some of the conditions requisite for their activity in soils are known, but on this phase of the subject there are many gaps in our knowledge and much work must yet be done before we can state definitely the part they play in the economy of nature, and before we can say which are the very best methods for increasing their usefulness.

Several investigators have tried to measure the part played by nonsymbiotic nitrogen-fixing micro-organisms in maintaining the nitrogen of the soil. Hall found gains of at least 25 pounds, Löhnis 37.7 pounds, and the senior author from 25 to 35 pounds per acre annually. The increase was much greater when suitable organic material was added. It is, therefore, conservative to state, with Lipman, that these organisms under favorable conditions add from 15 to 40 pounds of available nitrogen to each acre of

soil yearly. If the nonsymbiotic nitrogen fixers were adding 15 pounds per acre yearly to the 375 million acres planted to harvested crops this would represent an annual gain in nitrogen of 2.8 million tons. If by better cultural methods associated in some cases with inoculation the annual fixation can be raised to 40 pounds per acre there would be a gain in nitrogen of approximately 7.5 million tons.

CHAPTER XX

NITROGEN FIXATION, SYMBIOTIC

THE farmers of ancient Rome observed that crops following legumes were better than those following nonlegumes. Even before the Christian era in the Far East legumes were used for green manure, and it has been the general experience of practical farmers throughout the ages that legumes increase the productivity of the soil. This knowledge has been crystallized in the slang phrase "too poor to raise beans!"

During the middle of the nineteenth century it was considered an established fact that plants could not use atmospheric nitrogen, yet practical experience and carefully conducted experiments pointed to the conclusion that the legumes possess peculiar powers. It is only within the memory of men now living that it has been learned that legumes are veritable nitrogen-fixing factories. It is to these that we must turn for our combined nitrogen, for in the words of Lipman: "At best nitrogen in commercial fertilizers can supply only a small part of the nitrogen requirements of crops. Energy costs may be reduced and superior catalysts may be developed in technical nitrogen fixation, but even then we shall have to depend on legumes, legume bacteria, and solar energy to give us combined nitrogen at the least cost. To that end we must not only seek to improve our farm practices as they apply to crop rotations, tillage, water supply, and the use of lime and mineral fertilizers, but we must search for more efficient types of legumes as well as more efficient types of bacteria, both symbiotic and nonsymbiotic."

Legumes.—It is the legumes which under appropriate conditions possess the valuable property of feeding on atmospheric nitrogen. They have certain characteristics by which they may be distinguished from the nonlegumes: (1) They produce their seeds in a pod. (2) Their flowers resemble a butterfly and are practically identical in the parts that form them. True, there is a variation in the shape and size of the parts as well as in the whole blossom, but a small flower picked from a head of red clover or a cluster of alfalfa blossoms shows the same characteristics.

(3) Their leaves also show an interesting resemblance, each leaf being usually composed of leaflets arranged along a common stem. (4) Under normal conditions the legumes have on their roots round or clublike growths of various shapes and arrangements. (5) The seeds of the leguminosae, as compared with those of the cereal grains, are rich in reserve protein.

How the Nitrogen is Fixed.—Only legumes which have nodules on their roots have the power of fixing nitrogen. These are small wartlike protuberances and are the homes of the nitrogen fixers. Nodules vary in shape and size according to the legumes on which they occur and the soil in which they are found. Those found on the bean plant are usually round; those on the clover, oval; those on the soy bean, large and round; while those on the alfalfa are small club-shaped, and often grouped in finger-like bunches. They are large and numerous in well-aerated soil. In water-saturated or tight soil they occur near the surface and are sometimes colored similar to a sun-burned potato. While the plants are forming seeds most of the nodules are soft and the internal tissue sloughs off, leaving the more resistant outer tissues as a mere shell which later decays.

If one of the nodules is cut open and the inner part examined with a high-power microscope the symbiotic bacteria may be seen. These are minute rodlike organisms which may take on the form of stars, crosses and other grotesque shapes, depending upon the plant from which they come. Each nodule contains billions of these organisms. They get into the roots of the young, partly starved plants. As the tips of the root hairs of the plant push out into the soil, they chance to come into intimate contact with the bacteria. They gain entrance, grow, and rapidly multiply, until finally a nodule is formed. They obtain their food from the plant juices. The plant gets its nitrogen from the bacteria growing in the nodules. Here we have two friends living together—each helping the other, each performing the task which it has become best suited to do through ages of specialization. The plant with its extensive specially constructed leaf surface drinks in the carbon dioxide and mysteriously gathers up the heat and light waves. These enter the cell (the laboratory of that master chemist, chlorophyll) where they are transformed into carbohydrates. This fuel is silently passed down to those tiny dynamos, the micro-organisms, which fill the nodules on the root. Here the carbohydrates are burned, and the resulting energy is used to cause the atmospheric nitrogen to combine

Fig. 63.—Root nodules caused by nitrogen-fixing bacteria. A, Crimson clover nodules; B, alfalfa nodules; C, lima-bean nodules; D, cowpea nodules; E, Canada-field pea nodules. (*After Kellermann.*)

with hydrogen and oxygen. Some of the resulting product is used in the construction of more bacterial cells. Most of it is passed on to the plant and becomes a part of its tissue and later

its seed. The bacteria probably receive their fuel in the form of a soluble carbohydrate, but we do not know the medium of exchange used in payment. It may be a nitrite, or nitrate, an amino-acid, or even a protein. We do know that it is a nitrogen-bearing substance which can be used by the plant in its constructive metabolism.

The Causative Organisms.—The legume bacteria, *Rhizobium leguminosarum*, are rods, and when full-grown vary in length from 1 to 4 or even 5 μ. It is not uncommon to find them

Fig. 64.—Bacteroids from a very young nodule of pea. (*After Burrill and Hansen.*)

from 0.5 to 0.6 μ wide and from 2 to 3 μ long. Some have been found to measure only 0.18 μ in width and 0.9 μ in length. They have a great tendency to form involution forms, especially in the older nodules, appearing as stars, crosses and various grotesque forms. The organisms do not stain readily with the ordinary aniline stains. Carbolfuchsin and aniline-gentian-violet are the most satisfactory. The former brings out the vacuolated condition especially in the bacteroids. All nodule bacteria are motile, but the mode of flagellation varies. They do not grow well on the ordinary laboratory media, nor do

they readily fix nitrogen away from the host plant. Gains of from 2 to 3 mg. of nitrogen per gram of mannite are about the maximum. However, they must be able to function to a limited extent in soil, for they have been found in soils years after the disappearance of the legume. It is quite possible that during this time they live in symbiosis with various simple plants which flourish in the soil.

Seven different varieties of legume organisms are recognized. These are classified according to the particular group of legumes that they invade:

1. Those which infect red clover, mammoth red, alsike, crimson, Egyptian, or white clover.
2. Those which infect alfalfa, sweet clover, bur clover, yellow trefoil, or fenugreek.

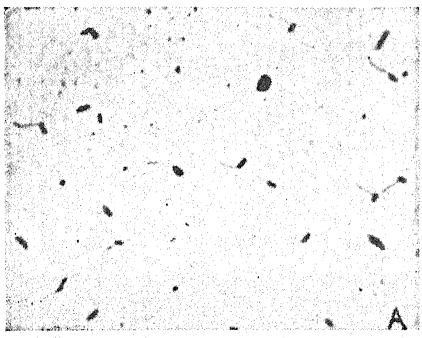


Fig. 65.—Rhizobium leguminosarum from nodule of cowpea. (*After Burrill and Hansen.*)

3. Those which infect the garden pea, hairy vetch, spring vetch, wild vetch, broad bean, lentil, sweet pea, or perennial pea.
4. Those which infect cowpeas, partridge pea, peanut, Japanese clover, velvet bean, lima bean, wild indigo, or tick trefoil.
5. Those which infect the garden, navy, kidney or scarlet-runner bean.
6. Those which infect lupine, serradella, or wild lupine.
7. Those which infect the soy bean.

Cross inoculation occurs from one plant to another within the same group, and infected soil or organisms may be successfully used within the groups.

Conditions Favoring Growth.—The legume bacteria are all aerobic and the nodules on the roots of the plants are usually

near the surface. The nodules will also form on plants grown in water cultures, but they are not as large and active as when grown in a well-aerated soil. Moreover, the legumes obtain their nitrogen through the roots and not the leaves; hence, the influence of cultivation on legumes is threefold: (1) The loosening of the soil brings atmospheric oxygen and nitrogen in contact with the nodule bacteria. (2) It increases the activity of decay bacteria which render essential plant food available to the higher plants. (3) The loose aerated surface tends to conserve soil moisture which can be drawn on as needed by the plant.

The root system of plants varies greatly with the moisture content of the soil. It has been found that legumes growing in moist soil have roots which spread widely, are high in water, are covered with root hairs, and present a large surface of young tissue—all conditions that are ideal for the growth of nodules large numbers of which are found on such plants. In dry soil the roots spread less and the epidermis is greatly thickened, consequently the tubercles are few and small on such plants.

The leguminosae require the same elements for their growth as do other plants and probably in addition small quantities of boron. Soils rich in available phosphorus and potassium, or fertilized with these constituents, produce numerous large active nodules if other conditions are ideal. Large quantities of available nitrogen, however, discourage the growth of the legume bacteria.

Most cultivated plants do best in an almost neutral soil reaction $p\text{H} = 7$. This is also true for the legumes, and where there is either excess alkali or acidity in a soil it must be brought to the right reaction for maximum growth.

They do best in a neutral soil, yet they will tolerate some acid, the quantity varying with the different organisms. The extent to which the different legume bacteria will tolerate acidity may be seen from the following pH values obtained by Fred and Davenport:

Rhizobia	*Critical* pH.
Alfalfa and sweet clover	4.9
Garden pea and vetch	4.7
Red clover and beans	4.2
Soy beans	4.2
Lupine	3.2

Influence of Nodules on Plant.—The nodules make it possible for the legume to utilize the atmospheric nitrogen. Soils poor

in nitrogen but otherwise suited to the growth of legumes can be made to produce excellent crops if properly inoculated and much larger yields are often obtained from inoculated than from uninoculated legumes. Legume crops bearing nodules on their roots are richer in protein and more valuable as a food than is a crop of the same legume which has borne no nodules. The seeds of the inoculated legume are usually superior to those of the uninoculated.

For centuries it has been the practice in China, Japan, Western Asia, and Northern Africa, to grow legumes and nonlegumes in combinations, and there is no doubt but that time and again practical farmers have noted the more vigorous growth and

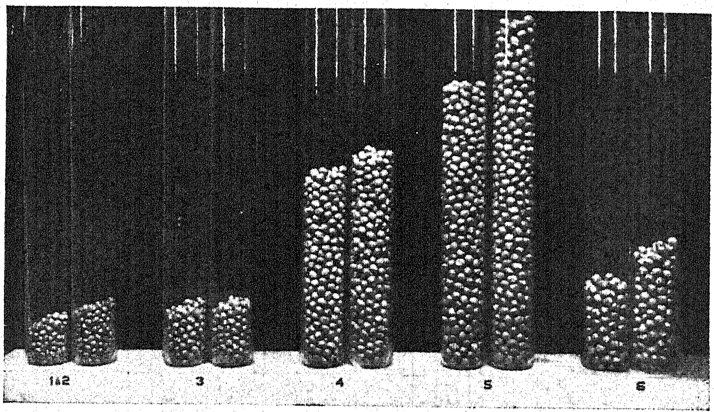


Fig. 66.—Influence of inoculations on yield of peas. (*After Fred, Whiting and Hastings.*)

darker green of nonlegumes when so grown. The discovery of the ability of properly inoculated legumes to fix nitrogen made it seem likely that they were assisting the nonlegume in obtaining atmospheric nitrogen. That this is actually the case was first demonstrated by Lipman. He grew nonleguminous plants in a porous pot surrounded by earth in a larger glazed earthenware pot in which leguminous plants were growing and found that under favorable conditions nonlegumes associated with legumes may secure large quantities of nitrogen from the latter.

Inoculation of Legumes.—Legumes fix nitrogen only when properly inoculated, and many experiments have been conducted to learn how and when to inoculate. Some of the early attempts

failed because knowledge of the different strains of bacteria was lacking and improper methods of growing and distributing the bacteria were employed. These difficulties have been largely overcome, so that today reliable cultures are available from the government and various state experiment stations as well as commercial concerns. However, even these cultures have little

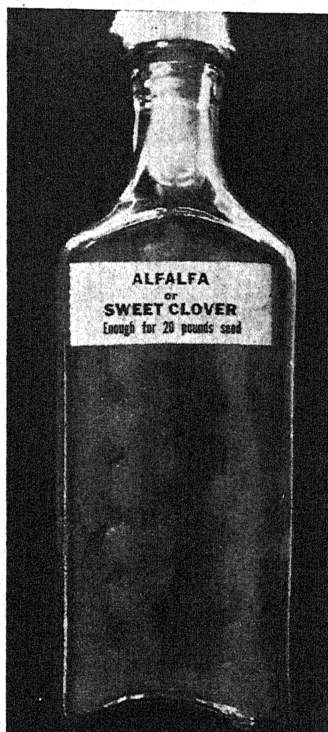


Fig. 67.—Bottle culture of root nodule bacteria. (*After Fred, Whiting and Hastings.*)

or no value unless other conditions are ideal, as for example: (1) Good physical conditions of the soil. (2) The soil must contain the elements essential to plant growth, with the exception of nitrogen. (3) The soil must be a suitable home for the legume and bacteria, that is, it must have a correct reaction, moisture, temperature, and aeration if maximum results are to be obtained.

It has been found that in the majority of soils where other conditions are favorable the legume bacteria are present. This is especially true where a particular legume has been grown for some time. The organisms may have been in the virgin soil growing on some native legume, or carried to the soil by various mechanical means.

When a legume is being grown on a piece of land for the first time, or even where considerable time has elapsed since legumes were grown, it may be necessary to inoculate. It is not definitely known how long legume bacteria will live in a soil. There is one case in which soy-bean bacteria have been known to live more than eighteen years in a fertile silt loam. Usually, however, the legume bacteria in a soil free from legumes decrease rapidly after two or three years. The only safe method is to make sure that the plants are inoculated. This can readily be done by digging up the roots and examining for the nodules. If plants from various parts of the field show a considerable number of nodules it is safe to assume that the plants are well inoculated.

It is argued by some that it is well to inoculate all legumes unless it is definitely known that the specific soil is well inoculated, for it is maintained that "cultures, when applied directly on the seed, enable the young roots to begin drawing nitrogen from the air as soon as the plants can use it. This early nodule formation is important, as it gives the plant a start which may influence the rate of growth to the extent that it gives a greater uniformity of maturity. In cases where some natural inoculation occurs, or where uneven soil inoculation is applied, the plant roots must grow through the soil until they come in contact with bacteria. This causes a delay in growth which may be disastrous, besides resulting in a serious unevenness in maturity, especially with soy beans and peas."

Gains in Nitrogen.—It has been found that from 50 to 100 pounds of nitrogen are gained to each acre depending upon the specific legume grown, extent of inoculation, and the conditions of the soil. In some exceptional cases gains even up to 200 pounds per acre yearly have been reported. Where a soil is well supplied with nitrogen the legume even though inoculated feeds mainly on the soil nitrogen, whereas in soils poor in nitrogen the legume is forced to make use of its ability to obtain free nitrogen from the air.

If only 10 per cent of the 325,000,000 acres of soil yearly producing crops were cropped to properly inoculated legumes it

would be a gain in nitrogen of over 8000 tons per year. The extent to which this would enrich the soil would depend upon the distribution made of the legume. If it were used as green manure the soil would be enriched to this extent. If fed and the manure carefully conserved and returned to the soil the soil gains in nitrogen would be from one half to three fourths this amount. However, where the legume is completely harvested and not even the manure returned, the gains in soil nitrogen would be small as the quantity in the roots and plant residues would not exceed that obtained from the soil. There are numerous cases on record where properly infected legumes have been used as green manures with average annual gains of soil nitrogen from 50 to 200 pounds. It is not as great where the legumes are fed and the manure returned to the soil, but, under this latter condition, the cost of the added nitrogen is considerably less.

CHAPTER XXI

THE PHOSPHORUS CYCLE

NITROGEN occurs primarily in the free form, phosphorus only in the combined. Nitrogen is very inert, phosphorus very active. The one tends to pass into the elementary form, the other into the complex. Bacteria are essential in the liberation of each from various organic compounds. Organic nitrogen they change into nitrates, elementary nitrogen into proteins; organic phosphorus they transform into the inorganic, the insoluble into the soluble. The nitrogen cycle is complex and has been extensively studied; the phosphorus cycle is probably less complex and has received less careful consideration.

Occurrence.—In comparison with some elements phosphorus is rare. It is twelfth in the order of abundance, as it constitutes approximately 0.14 per cent of the earth's crust. Even this is unevenly distributed. Some virgin soils contain enough for only a few crops, whereas others contain sufficient for hundreds of crops. It also occurs in large natural deposits. It exists primarily as phosphates both in the soil and in natural deposits. The phosphorus resources of the United States are superior to those of any other nation. Although it is well distributed both in the East and in the West, the bulk of it occurs in Utah, Idaho, Wyoming, and Montana. In these states are billions of tons of high-grade phosphate. The tendency is to mine and ship most of the highest grade to foreign countries. This is a wrong practice, because in the future it will be needed at home for the production of food for our ever-increasing population.

Next to nitrogen, phosphorus is the limiting element of crop production in most soils. Furthermore, the nitrogen supply is inexhaustible since it forms four fifths of the earth's atmosphere and can be obtained cheaply by straight processes of industrial chemistry, or, better still, by the nitrogen-gathering bacteria. On the other hand, from thousands of analyses Hopkins has calculated that the average supply of phosphorus in the 2,000,000 pounds of soil covering an acre of land to the usual plowed depth of 5⅔ inches is only 2200 pounds, or enough for 135 crops of corn at the rate of 100 bushels per acre.

Phosphorus occurs in every cell, both plant and animal. Nucleoproteins (which constitute such a large part of the cell nucleus) on hydrolysis yield nuclein, and nuclein contains approximately 10 per cent of phosphorus. It constitutes over 20 per cent of the ash of corn, 19 per cent of the ash of wheat, 12 per cent of the ash of barley, and over 9 per cent of the ash of oats; hence, there are millions of pounds of it in each yearly harvest. The mineral part of animal bones consists largely of tricalcium phosphate. In 100 pounds of raw bone there is approximately 10 pounds of phosphorus. An adult contains in his body about 1.5 pounds of phosphorus. If we allow one half of this amount for each individual it would mean, in round numbers, 700,000 tons of it tied up in the bodies of individuals living upon the earth at the present time, to say nothing of the far greater quantity bound up in the bodies of animals. Thus, it may be seen that phosphorus follows close to nitrogen after carbon, oxygen, and hydrogen as a structural material in biological chemistry.

Function of Phosphorus.—Phosphorus is an essential constituent of the microbial body and plays a vital rôle in all carbohydrate fermentation whether it be due to bacteria, yeast, or molds. It is present in every cell and fluid of the animal body and often not as mere structural material. It together with calcium plays a fundamental rôle in bone formation and enters into the buffering powers of the blood and other tissues. Phosphorus is vitally concerned with carbohydrate, fat, and some phases of protein metabolism. It is essential in muscular contraction and in the functioning of the central nervous system. The early workers were so impressed with this universality of phosphorus in the nervous system that they coined the aphorism: "Ohne Phosphor keine Gedanke" ("Without phosphorus, no ideas").

Outline of Phosphorus Cycle.—The mineral phosphates of the soil are rendered soluble through bacterial activities. This is taken up by the living plants and deposited either as organic or inorganic phosphorus within the plant tissues. The plant tissues, if eaten by an animal, yield phosphorus to the animal which is laid down in its body in the form of organic or inorganic compounds. The excreta of animals always contain phosphorus in both organic and inorganic forms. The inorganic phosphorus is readily utilized by plants and again starts on its cycle. However, the organic phosphorus in plant and animal residues must be mineralized by bacteria before it can be used by plants. Micro-organisms split off the carbonaceous material and phos-

phorus is liberated mainly in the form of phosphates. Under some conditions mold action may give rise to small quantities

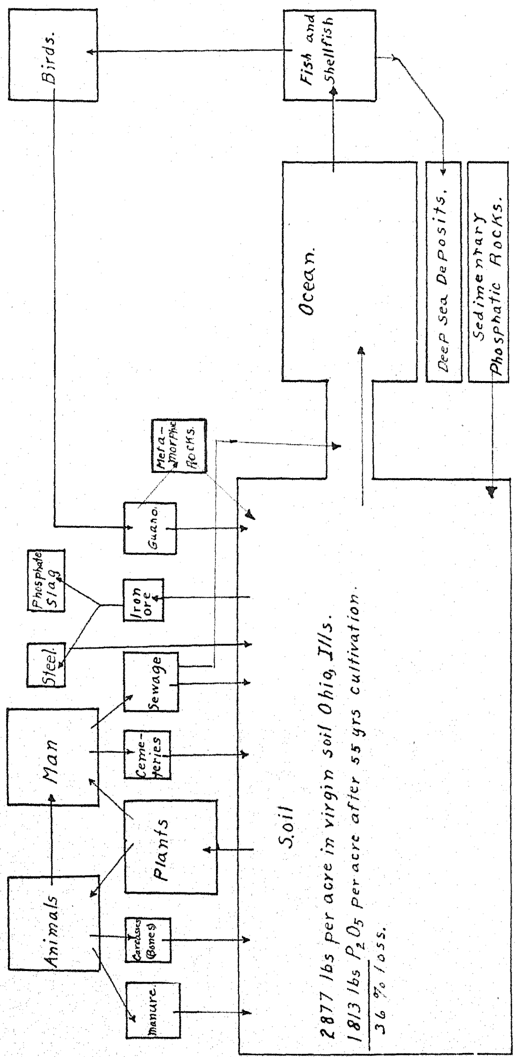


Fig. 68.—Illustrating the phosphorus cycle in nature. (*After Lotka.*)

of phosphine which must be oxidized again before becoming available to higher plants. In either event, the resulting phosphate is now ready to start once more on its cyclic journey through

plant and animal organisms which is dramatically portrayed by Martin as follows:

"I (a phosphorus atom) have a dim recollection of looking out from a cliff face upon a widespread blue sea, filled with strange vast monsters, which have long since vanished from the earth. But at last the cliff was washed away and I passed into the great body of the sea, and was absorbed into a tiny plant, living beneath the salt waters; but this was devoured by a glittering gorgeous fish, and so I entered his body. Then the fish was devoured by a reptile, which, creeping out of the water, entered a swamp and died, and its huge body decaying, I was washed into the soil, and there meeting the rootlet of a plant, I entered into and formed part of it; and this was eaten by an animal; and so I entered into its body and formed part of its bones. While we were crossing a ravine one bright sunshiny day, millions of years ago, a green monster flashed upon me and slew my master and devoured me. After a time my new host was also slain in a similar manner, and his body, decaying in the rank grass and vegetation of the swamp, I was ultimately washed out to sea in a sudden flood, which coming down from the hills swept me away . . . One day, I was hurled forth amidst vast thunderings through the throat of a great volcano, and formed part of a molten lava stream, which in time became a fertile field covered with waving crops and golden grain. Then I entered into a grain of corn, and was devoured by a man living thousands of years ago, a mere savage you would term him, wild and fierce. From him I passed to earth once more, and since then have been passing in a ceaseless round of change through the bodies of living creatures. I have flown through the air in a bird, I have swum in the sea in a fish, I have roamed over the earth in a beast, I have formed part of innumerable plants . . . One day, a few years ago, I was devoured by an ox while part of a piece of grass, and soon by the mysterious chemical forces of its body I was made to form part of its bone. The great beast was slaughtered by men, and his flesh eaten, and his bones burnt to a fine white dust in a furnace. Out of this dust, I, the tiny phosphorus atom, was distilled in a furnace and found my way to a match factory, and am now in this little match-box lying on the table before you."*

Fermentation.—The fermentation of carbohydrates, with the production of alcohol, illustrates the importance of phosphorus in some vital phenomenon. The malt, prepared by a regulated arti-

* From "Triumphs and Wonders of Modern Chemistry" by Geoffrey Martin, Sampson Low, Marston and Co., Ltd., Publishers.

ficial germination of barley, contains a number of enzymes. Some of the more important are: An oxidase functioning chiefly as an activator in connection with the respiration of the growing plant; a cytase which attacks the walls of the starch-containing cells, making them permeable to the amylase, which penetrates and converts the starch into dextrine and maltose; a proteolytic enzyme which hydrolyzes portions of the complex insoluble proteins into simpler soluble derivatives; and a phytase which liberates the phosphorus that plays a vital rôle in the fermentation of the wort.

The germinated grain is slowly dried, first at a low temperature then at a higher. The high temperature assists in developing color, aroma, and flavor. Malt, water, and yeast are mixed, kept under well-aerated condition at an optimum temperature so that the sucrose, maltose, dextrose, and levulose may be converted into alcohol. It is usually assumed that the disaccharides are first hydrolyzed into hexoses by the enzymes sucrase and maltase. Dextrose and levulose are both fermentable by zymase, levulose being fermented more rapidly. There is some evidence that all the hexoses are first converted into levulose by an unknown enzyme and then fermented. The stages in the fermentation, together with the function of phosphoric acid are given by Meyerhoff as follows:

INITIAL PHASE

"1 dextrose + 1 hexose diphosphoric acid + 2 phosphoric acid → 4 triosephosphoric acid → 2 glycerophosphoric acid + 2 phosphoglyceric acid.

"2 phosphoglyceric acid → 2 pyruvic acid + 2 phosphoric acid → 2 acetaldehyde + 2 carbon dioxide + 2 phosphoric acid.

STATIONARY PHASE

"1 dextrose + 2 acetaldehyde + 2 phosphoric acid → 2 triosephosphoric acid + 2 acetaldehyde → 2 alcohol + 2 phosphoglyceric acid."*

Phosphorus plays a similar rôle in lactic acid production, and it is even probable that it enters into many, if not all, fermentative changes.

Mineralization of Phosphorus.—Plant and animal residues, together with micro-organisms, contain appreciable quantities of nucleoproteins, phosphoproteins, lecithins, phytins, and other

* From Meyerhoff in "Nature" 132, 375.

phosphorus-carrying complexes. These must be broken down (mineralized) by micro-organisms, with the liberation of their phosphorus, before it can be utilized by higher plants.

A number of common soil organisms decompose nucleoproteins into amino-acids, carbohydrates, phosphoric acid, purine and pyrimidine bases. The probable steps are: The nucleoproteins are first decomposed into a simple protein and nuclein; the nuclein is further broken into a simple protein and nucleic acid; the nucleic acid is decomposed by other bacteria into carbohydrates, phosphoric acid, purine and pyrimidine bases.

The genus nucleobacter is especially concerned with the decomposition of nucleins, through the nucleic-acid stage into phosphoric acid. Schittenhelm has shown that nearly all of the nuclein substances of feces disappear as they undergo autoputrefaction. He and Schroeter showed that bacteria may bring about a deep cleavage of yeast nucleic acid. Plenge demonstrated that some bacteria have the power to liquefy the sodium salt of nucleic acid.

Bacteria act upon lecithin hydrolyzing it into glycerin, two molecules of fatty acid, choline, and phosphoric acid. The large quantity of phosphorus in phytin is readily liberated as inorganic phosphorus by a number of molds and bacteria which produce the enzyme phytase. The phosphorus liberated from these organic compounds may be taken up by the growing plant; however, often much of it is built into the body of micro-organisms, and as they die the phosphorus is rendered available by still other micro-organisms.

Solvent Action of Bacteria.—The insoluble phosphorus of the soil may be rendered soluble by micro-organisms in three ways: (1) By the direct metabolism of micro-organisms, which may be due to enzymes or there may be a reaction between synthetic bacterial substances and the insoluble phosphorus, resulting in a soluble organic or inorganic compound of phosphorus; (2) by the action of carbon dioxide and various organic acids produced by the micro-organisms; (3) by the production of inorganic acids, nitrous, nitric, and sulphuric acids, by the autotrophic bacteria.

Grazia considers enzyme action to play a part in the dissolving of phosphates in soil. He found that the addition of chloroform to a soil reduces bacteria and the acids produced by them yet increases solution of phosphates. It is also known that the presence of ammonium chloride and sulphate in the cultural media is especially effective in increasing the solvent action of bacteria. This may come from the organisms removing the am-

monia as a source of nitrogen and leaving the sulphate and chloride ions in the medium. Other workers have also reported the production of soluble phosphates due to the vital function of the micro-organism, whereas still others question the existence of specific enzymes capable of bringing into solution insoluble phosphates. However, it is certain that micro-organisms may assimilate the small quantities of comparatively insoluble phosphates which pass into solution and concentrate it within their bodies, the resulting phosphorus-carrying compounds being readily decomposed by still other bacteria.

There are reported cases in which bacterial activities have decreased the water-soluble phosphorus of the soil and of raw rock

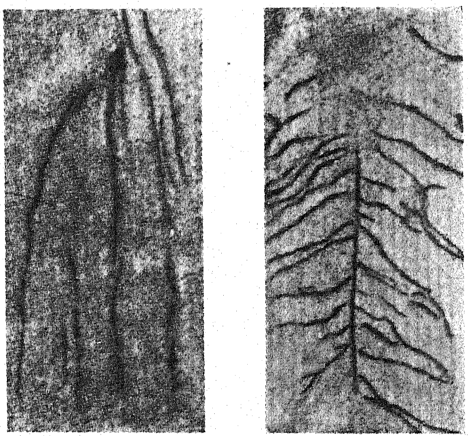


Fig. 69.—The picture on the left shows the solvent action of plant roots on a marble slab in the absence of bacteria; the one on the right the action in the presence of bacteria. (*After Fred and Haas.*)

phosphate. This does not mean, however, that it is less available; for, as pointed out by Truog, the mixing of floats with manure causes an immediate decrease in the solubility of the phosphorus in 0.2 per cent citric acid solution. Yet when such a mixture is thoroughly incorporated into the soil within feeding area of the plants, the availability of the phosphorus is increased to such an extent that some species of plants are apparently able to secure almost an adequate supply of it from the floats. The addition of manure to a soil greatly increases the carbon dioxide production and for a short time measurably increases the solubility of the phosphorus. The decrease in solubility comes later,

probably due to the formation of nucleoproteins within the bodies of the bacteria which still later may be rendered soluble either by further bacterial activity or by autolytic enzymes.

Many soil micro-organisms generate large quantities of carbon dioxide. Azotobacter, for example, when growing under appropriate conditions, possesses the power of producing 1.3 times its own body weight in carbon dioxide during twenty-four hours. Waksman states that 1 Gm. of bacterial cells produces 0.25 to 0.5 mg. of carbon dioxide in twenty-four hours and that 1 Gm. fungus mycelium produces 0.13 to 0.18 mg. carbon dioxide. As much as 6000 pounds of carbon dioxide may be given off in 200 days by 1 acre of normal soil. Water charged with carbon dioxide is a universal solvent and will attack even ordinary quartz rock. Granite and rocks related to it are also quite readily attacked, with the liberation of potassium and other elements. Carbonated water acting upon the tricalcium phosphate of the soil would form more readily soluble phosphates from tricalcium phosphates.

The oxidation of ammonium salts to nitrous acid by the *Nitrosomonas* and its further oxidation to nitric acid by the *Nitrobacter* give rise to strong mineral acids which are certain to act as soil solvents. Hopkins and Whiting found that neither ammonifiers nor true nitrifiers liberate appreciable quantities of soluble phosphorus. However, they consider the nitrite bacteria of fundamental importance in rendering phosphorus and calcium soluble by the oxidation of ammonia into nitrous acid which reacts with the raw rock phosphate, thereby rendering it soluble. The actual ratio found was about 1 pound of phosphorus and about 2 pounds of calcium for each pound of nitrogen oxidized. In addition to the acid radicles associated with and resulting from the oxidation of the ammonia, carbonic acid plays an important rôle. These acids may play a great part in soils low in calcium carbonate, but Kelley has shown that in those rich in calcium carbonate there are only small quantities of phosphorus liberated.

The transformation of rock phosphate into soluble forms by sulphuric acid generating micro-organisms is similar to the change which occurs with nitrous acid. In pure cultures, or in composts, the transformation of the phosphate is rapid and almost complete. In the soil, the sulphuric acid tends to react with the calcium and magnesium carbonates, silicates, and salts of organic acids in preference to phosphates.

Moreover, soil organisms form, among other products, formic, acetic, lactic, butyric, and other acids, the kind and quantity of

each depending upon the specific micro-organisms and upon the substance on which they act. These substances are certain to come in contact with some insoluble plant food which they may render soluble. This may be concentrated by the soil microbes in their bodies which are especially rich in phosphorus. The phosphorus, on the death of its host, is probably returned to the soil in a readily available form, for it must not be forgotten that, although many of the organic phosphorus constituents may not be soluble in pure water, they may be more available to the living plant than are the constituents from which they were first derived through bacterial activities.

Biological Test for Available Phosphorus.—Bacteria are being used to determine the phosphorus deficiencies of a soil. A series of samples of soil are mixed with starch and varying quantities of phosphate. The soils are then inoculated with Azotobacter, made into a paste with water, and placed into dishes for incubation. Where conditions are favorable Azotobacter grows well and forms on the surface tiny pearl-like colonies. If conditions are unfavorable, the colonies are few or absent altogether. It is self-evident that all other conditions except phosphorus must be kept at an optimum in order that the test have diagnostic value.

Losses of Phosphorus.—When rendered soluble, phosphorus may be (1) taken up by the growing plant, (2) reverted to insoluble phosphates and hence remain in the soil, or (3) carried out by the drain waters into rivers, lakes, or oceans. As the plants are eaten by the lower animals, the major portion of the phosphorus is returned to the soil. This is not true of that consumed by man, since most of it finds its way into the sewer. Van Hine estimates that the annual loss of phosphorus from this source is about 176,000 tons. The quantity annually locked up in the bodies of individuals who die in the United States is estimated by Lotka as 1105 tons. The quantity which is lost through leaching is even greater, as Blackwelder writes: "Of the vast quantity of mineral matter annually delivered to the sea by the runoff, it is estimated that 1.45 per cent consists of phosphorus pentoxide. Using the best available figures for the amount of water thus brought to the ocean annually, it is calculated that if the phosphatic material in the form of solid tricalcium phosphate were loaded into standard railroad cars it would fill a train stretching continuously from Boston to Seattle and would be 7 to 12 times as great as the world's total production of phosphatic rock in 1911."

When the phosphorus reaches the ocean, nature begins its con-

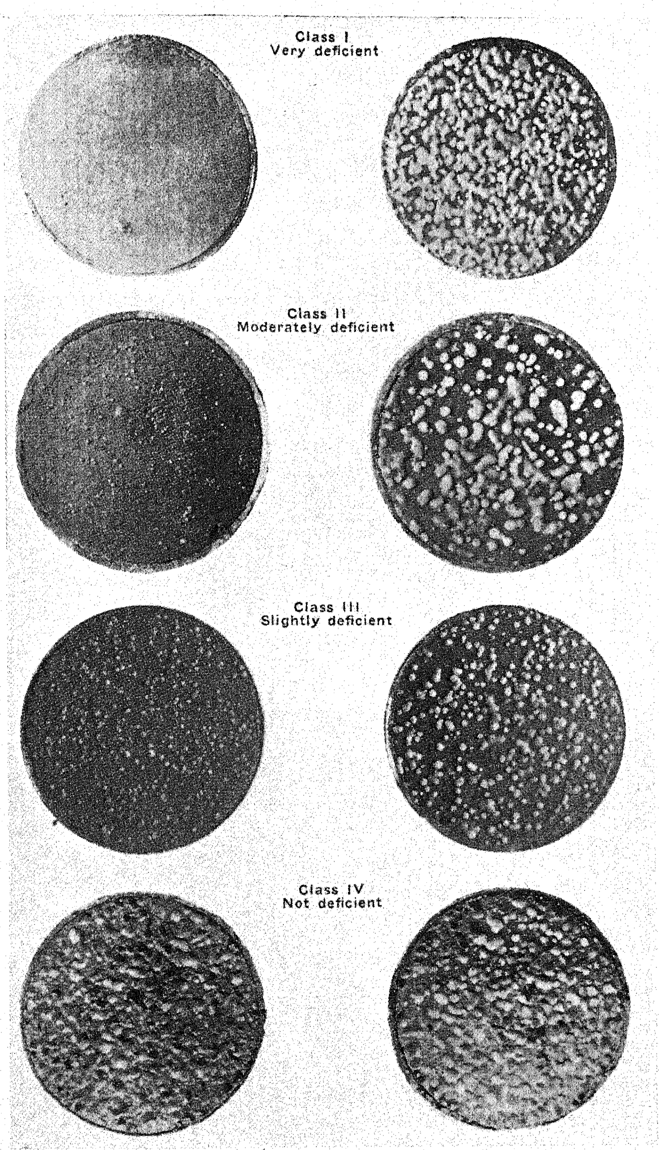


Fig. 70.—Type plaques for phosphate deficiency classification. Left row: Checks, nothing added; right row, phosphate added. (*After Sackett and Stewart.*)

centration. "In the sea water blue-green Algae concentrate phosphorus. Certain mollusks or crustacea feed on the Algae, and other meat-eating mollusks devour the vegetarians. Small fishes eat the mollusks, large fish eat the small, finally seals and birds swallow the fishes, and so on in about six transformations the phosphorus originally contained in the sea water may come to rest in deposits of guano on desert islands or in accumulations of bones of vertebrate denizens of the sea." After concentration terrestrial disturbances may elevate it above the water where it may be mined by man and used as a fertilizer on his soil.

CHAPTER XXII

THE SULPHUR CYCLE

THE sulphur and nitrogen cycles have much in common. Both of these elements journey through earth, air, and water. Native nitrogen occurs in the atmosphere, native sulphur in soil and water. Both find their way into the atmospheres through combustion and decay. The supply in the soil comes from the atmosphere, but the quantity and nature of the products in the two cases are far different. Nitrogen occurs in the atmosphere in limitless quantities and can be used by the plant only after bacteria have caused it to combine with other elements. Sulphur is there in only traces. The rains wash it into the soil where it is soon rendered available to the growing plant. When the supply in the soil of either of these elements becomes insufficient, they must be added in the form of fertilizers.

Occurrence of Sulphur.—Sulphur constitutes 0.08 per cent of igneous rock. It is estimated that 0.12 per cent of the earth is sulphur, where it occurs in both the free and combined form. Large deposits of free sulphur are found in Sicily, Japan, and in Louisiana, Texas, and other parts of the United States. Soil contains it in rather small quantities both in the organic and inorganic forms. Inorganically, it occurs mainly as sulphates combined with calcium, magnesium, sodium and other common bases. Its origin is the native rock, organic manures, and rain water. It is emitted by volcanoes, and sulphur springs contain it in large quantities.

Sulphur is one of the essential elements of plant food. It is necessary for the formation of certain essential oils and enters into the composition of the bodies of all plants and animals. The quantity is small and varies with different plants, as may be seen by the results of Hart and Peterson shown on page 242.

It has been found at the Utah Experiment Station that the quantity in grains increases with the irrigation water applied during their growth. This increase is mainly inorganic sulphur; hence, it would have no food value to higher animals, inasmuch as they can use only organic sulphur.

Crops	Size of crop per acre	Sulphur in plants	
		Per cent	Lbs. in crop
Alfalfa hay	8 tons	0.287	45.92
Red clover	4 tons	0.164	13.12
Corn grain	100 bushels	0.170	9.52
Corn stover	3 tons	0.126	7.56
Oats	100 bushels	0.189	6.05
Oat straw	2.5 tons	0.195	9.75
Timothy	3 tons	0.190	11.40
Wheat	50 bushels	0.170	5.10
Wheat straw	2.5 tons	0.119	5.95

Properties of Sulphur.—All have seen the familiar yellow sulphur either as brimstone or as the flowers of sulphur. It is without a marked taste and possesses only a faint odor. It is insoluble in water; when heated it melts, the nature and consistency of the product depending upon the temperature. Its great value resides in its ability to form compounds with other elements. It combines with oxygen yielding gaseous disinfectants. It forms strong acids which readily react with basic substances, thus yielding available plant food in the soil; when oxidized, it yields energy which may be used by certain micro-organisms. Hence, we find bacteria continually at work changing it from the gaseous to the solid and from the solid to the gaseous form.

Outline of Cycle.—Sulphur is a constituent of the bodies of plants and animals, and as these putrefy or decay it finds its way into the atmosphere. The combustion of coal yields large quantities. Enormous quantities of hydrogen sulphide and sulphur dioxide are emitted by volcanoes. These substances may be oxidized and washed from the atmosphere in the rain water. In the soil the products are worked over by bacteria and combine with the bases to yield sulphates. These may be taken up by the growing plant or leached out by waters and wander to the sea. "The quantity of sulphates discharged into the sea is enormous, and we may well wonder why the sea is not mainly a sulphate solution. As it is, it contains in the dissolved salts 7.69 per cent of the sulphate radical. The reason is that it is being continually reduced to sulphides. These react with iron with the production of iron sulphide. Evaporation of the sea water or lake water in closed basins precipitates calcium sulphate at an early stage and there results our deposits of gypsum. From these, possibly

through bacterial action, there results sulphur. A large part of the sulphur of the world is then continually in movement, changing from sulphide to sulphate with local reversion to native sulphur and from sulphate back to sulphide again."

The sulphur taken up by the growing plant is built into the plant structure, later decomposed by the animal, or becomes a part of its tissue. The sulphur of the plant and animal tissue may be liberated through combustion or by bacterial action and again start on its journey.

Rôle of Sulphur Bacteria in Early Geological History.—Bacteria which possess the power of assimilating carbon dioxide in

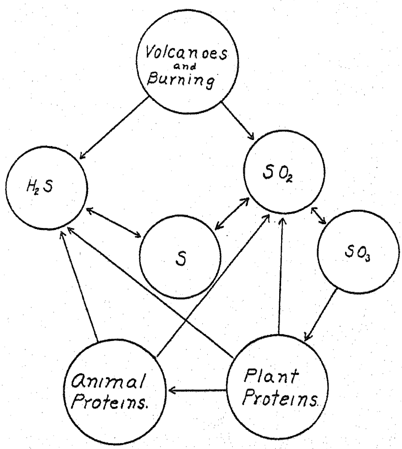


Fig. 71.—Illustrating the sulphur cycle in nature.

the absence of sunlight are known as autotrophic bacteria. The energy necessary for this assimilation is obtained from the oxidation of simple inorganic substances. The nitrite, nitrate, and certain sulphur bacteria are the only bacteria truly autotrophic. The methane, hydrogen, and especially the iron bacteria, while they possess the ability to obtain their energy from these simple compounds, may also use more complex organic substances as a source of energy. These are known as heterotrophic bacteria.

The sulphur bacteria, then, like the nitrifying bacteria, can live upon the bare rock. They obtain their carbon from the atmosphere and function best in a concentration of carbon dioxide greater than is found in the atmosphere today. They must have found ideal conditions in the heavily laden atmosphere

of early geological periods. They obtain their nitrogen and energy from the ammonia and sulphur brought to them by the rain waters. They oxidize sulphur to sulphuric acid which reacts with the rock constituents and renders them available. Investigations clearly indicate that the autotrophic bacteria can live on very simple inorganic compounds and build from them complex organic materials which later may be used by other organisms. It is quite likely that the sulphur bacteria, together with other autotrophic micro-organisms, were the pioneers which gained a precarious foothold on the bleak, primitive rock and rendered it a fit abode for higher plants and animals.

Sulphur Bacteria.—The so-called "sulphur bacteria" have been divided into five groups which vary widely morphologically and physiologically:

1. Colorless, thread-forming bacteria, which accumulate sulphur within their cells. They consist of three genera:

1. Beggiatoa—motile, forming no sheaths.
2. Thiothrix—fastened, forming no sheaths.
3. Thioploca—thread-forming bacteria, surrounded with a jelly-like sheath.

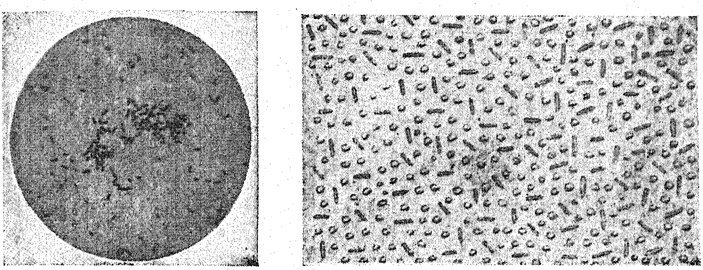
These bacteria oxidize hydrogen sulphide and accumulate sulphur in the form of small spheres, consisting of soft amorphous sulphur which never crystallizes in the living cells. The sulphur is oxidized to sulphuric acid, which is immediately transformed into sulphates by the carbonates present. In the absence of sulphur, the organisms soon die. They require oxygen and ammonium salts but function in the absence of organic matter.

2. Colorless, nonthread-forming bacteria, accumulating sulphur within their cells. There are several members of this group which have been studied. They differ from the members of the first group in that they do not form threads. They are found in the muds and sulphur water.

3. Purple bacteria, oxidizing sulphur and accumulating it within their cells. They are distinguished from the other sulphur organisms by a red, reddish-violet or reddish-brown pigment which is unevenly distributed throughout the cell. In addition to the red pigment (*bacteriopurpurin*), they also contain a green pigment (*bacteriochlorine*). They are found abundantly in sulphur springs and in mud waters.

4. The fourth group of sulphur bacteria includes colorless organisms that do not accumulate sulphur within their cells.

The two members of this group which have been studied are *Thiobacillus thioparus,* an aerobic organism, and *Thiobacillus denitrificans* which is an anaerobe. They act on hydrogen sulphide and the sulphides with the production of free sulphur which never accumulates within the cell, although an abundant quantity accumulates outside and not in direct contact with the cell.



a *b*

. Fig. 72.—*a,* Thiobacillus thiooxidans; *b,* Thiobacillus thioparus, showing drops of precipitated sulphur among the rod-shaped organisms. (*From "Principles of Soil Microbiology," by S. A. Waksman, Williams and Wilkins Co., Publishers.*)

5. The fifth group oxidizes elementary sulphur and does not accumulate any sulphur either within or without the cell. The only member of this group which has been so far studied is *Thiobacillus thiooxidans.*

This organism was first obtained in pure culture by Lipman and co-workers by repeatedly transferring it to fresh media having a high acidity (*p*H 2.0–3.0). Later Waksman grew it on a solid medium of the following composition:

Sodium thiosulphate	5.0 gram
Monopotassium acid phosphate	3.0 gram
Ammonium chloride	0.1 gram
Magnesium chloride	0.1 gram
Calcium chloride	0.25 gram
Agar	20.00 gram
Distilled water	1000.00 cc.

It is a short rod with rounded ends, usually occurring singly, occasionally in pairs, but rarely in triplets. The majority of these organisms are less than 1 μ long and about 0.5 μ in diameter.

Spore formation does not take place. The majority of the cells are nonmotile. The organism stains well with gentian violet and methylene blue, and it is gram-positive.

This micro-organism is of especial interest for the following reasons:

1. It, like other autotrophic bacteria, can utilize directly the carbon dioxide of the air. The energy comes from the oxidation of elementary sulphur.
2. It functions in the complete absence of organic food.
3. It is able, not only to tolerate, but to produce higher concentrations of acid than any other known living organism.

Sulphur Oxidation in Soil.—Whenever sulphur finds its way into a normal soil, it is slowly oxidized to sulphuric acid. But when this same soil has been inoculated with *Thiobacillus thiooxidans,* the sulphur is rapidly oxidized directly to sulphuric acid. The acid produced acts upon insoluble soil constituents such as calcium and magnesium carbonate, calcium silicates, and tricalcium phosphate and brings them into solution. Under appropriate conditions the reaction may render available appreciable quantities of plant food.

The ratio between the sulphur oxidized and the carbon assimilated is about 32, but this ratio varies widely, depending on the conditions of growth. The variation is larger in the presence than in the absence of nitrates and glucose. When thiosulphate is used as the source of energy, the ratio is about 65. About 6.5 per cent of the energy made available is utilized by the organisms. This is a somewhat greater efficiency than is obtained with the nitrite and nitrate bacteria, for the sulphur bacteria are more efficient engines than most living cells.

The sulphur of the plant and animal residues which finds its way into the soil is liberated by bacteria as sulphur dioxide, hydrogen sulphide, or elementary sulphur. These compounds in turn are transformed by the various sulphur organisms into sulphates and made available for the use of the higher plants.

Soil Losses of Sulphur.—The end-products of the sulphur cycle in soils are sulphates, all of which are soluble. These may be removed from the soil by the growing plant or by drain water. The quantity removed by the plant depends upon the kind and size of crop grown on the soil. Alfalfa, cabbage, turnips, and onions remove comparatively large quantities of sulphur, whereas the grains, corn, oats and wheat remove smaller quantities. This may be seen by the following table of Hart and Peterson:

SULPHUR REMOVED BY DIFFERENT CROPS

Crop	Dry weight, pounds	Sulphur removed, pounds
Alfalfa	9,000	26.0
Red clover	3,763	6.2
Sugar beets		
Roots	4,320	3.8
Tops	1,848	8.0
Total crop	5,168	11.8
Turnips		
Roots	3,126	23.1
Tops	1,531	13.8
Total crop	4,557	36.9
Cabbage	4,800	39.2
Corn		
Grain	1,500	2.6
Stalks	1,877	2.2
Total crop	4,377	4.8
Oat		
Grain	1,625	3.0
Straw	2,353	4.9
Total crop	3,978	7.9
Wheat		
Grain	1,530	2.7
Straw	2,653	3.7
Total crop	4,083	6.4

At this rate 400 pounds of sulphur in the soil would be sufficient for about 15 crops of alfalfa, 10 crops of cabbage, or 63 crops of wheat. It has been found that the soil of the Greenville Experimental Farm (Logan, Utah) contains only 247 pounds of total sulphur in the surface acre-foot. This would be sufficient to meet the needs of 39—50-bushel crops of wheat, 31—100-bushel crops of corn, or 38—75-bushel crops of barley grain at the rate these grains are at the present time actually taking the sulphur from the soil. It is doubtful whether the sulphur content of the average soil of the United States contains over 400 pounds per acre of sulphur. Hence, it is evident that the sulphur actually stored in

the soil at a given time is sufficient to produce crops for only about one generation of human beings.

The second and greater loss of sulphur comes from the leaching-out of the soluble-sulphur compounds by drain waters. Many factors influence this loss; namely, the kind of soil, the amount of precipitation, system of cropping, and methods of cultivation. The greatest loss comes during the season of the year when no crop is growing on the soil. This is augmented by everything which tends to loosen and aerate the soil. The actual loss of sulphur in some soils has been found to be as low as 8 pounds per acre yearly, but in others as high as 281 pounds. Some investigators have noted a loss of from 40 to 50 per cent of the total sulphur of the surface soil within a period of fifty years. The reduction would be even greater than this were it not for the fact that there is a compensating factor in the sulphur cycle.

Soil Gains in Sulphur.—Combustion and decay are constantly liberating gaseous sulphur compounds. Millions of tons of sulphur are thrown yearly into the atmosphere by volcanoes. This sulphur finds its way back into the oceans, lakes, streams and soils of the country. The quantity brought to the earth varies greatly in different localities. It is high near volcanoes and manufacturing centers where fuels rich in sulphur are used. Reports have been made of regions where the annual sulphur brought to the soil by rain water does not exceed 4 pounds per acre. However, in other localities as much as 131 pounds per acre is annually brought to the soil. The average yearly quantity brought to each acre of tillable soil is approximately 25 pounds. Were it not for this gain from rain waters the sulphur question would be more acute than the nitrogen problem.

Soils also gain sulphur from plant and animal residues. Each ton of barnyard manure carries to the soil about 3 pounds of sulphur and the quantity added in commercial fertilizers may at times be sufficient to actually increase soil sulphur in addition to replacing that removed by the growing plant and lost in the leach water.

Economic Consideration of Sulphur Cycle.—It is evident from what has been said that the sulphur organisms are essential for the life of higher plants and animals. Were it not for them, sulphur would be locked up in forms unavailable to higher life and eventually all of the available sulphur would disappear. However, before this could happen all forms of life would disappear, as sulphur is essential to the normal functioning of every cell.

Sulphur is oxidized to sulphuric acid by bacteria. The acid in

turn reacts with the various insoluble constituents of the soil and renders them soluble. By this means the phosphorus and potassium are made available to the growing plant. The Lipman process for preparing soluble phosphorus is based on this principle. Raw rock phosphate and sulphur are mixed with some inert substance, inoculated with *Thiobacillus thiooxidans* and kept at optimum moisture and temperature conditions. In time there results acid phosphate which in the future may replace the present commercial phosphate. In this manner bacteria and sulphur replace the sulphuric acid used in the commercial process.

Preliminary tests indicate that the sulphur bacteria may be made to play an important rôle in the reclaiming of black alkali soil. Sulphur added to the soil is oxidized to sulphuric acid. This in turn reacts with the highly toxic sodium carbonate and transforms it to the less toxic sodium sulphate which, if necessary, may be leached from the soil.

Preliminary experiments indicate that in some soils it is possible, by the addition of sulphur, to change the reaction of the soil to such a degree that the development of certain pathogens is prevented without injuring the particular plant. In some cases of field tests it has been found possible by this method to decrease potato scab from 30 to 60 per cent.

CHAPTER XXIII

BACTERIA IN RELATION TO OTHER ELEMENTS

PLANTS require ten or twelve elements for their normal growth and fruition. Animals require even more. These elements are built into complex compounds and hence become unavailable to other higher plants. We have learned how carbon, nitrogen, phosphorus and sulphur are transformed by bacteria, so that they again become useful to the higher plant. Let us now examine briefly the wanderings of a few of the other "life elements."

Iron.—Iron constitutes 5.5 per cent of the earth's solid surface. Aside from oxygen it is the most abundant of the "life elements" and is required by plants and animals in smaller quantities than is the case with any of the other elements. A 50-bushel crop of wheat contains one hundred times as much nitrogen; ten times as much phosphorus and eight times as much sulphur as iron. Yet small as the quantity of iron may be, it is absolutely essential to the life and well-being of plants and animals.

Although iron does not enter into the composition of chlorophyll, it plays an important part, directly or indirectly, in the production of chlorophyll; for, if iron is withheld from the plant, the leaves do not become green. Later, if the iron is supplied, the chlorophyll soon appears. In the animals, iron plays a vital part. In the body of the human adult there are about 4.5 Gm., contained for the most part, in the hemoglobin of the red corpuscles. The importance of iron arises from the fact that it plays the essential rôle of an oxygen carrier. It acts as a catalyst causing the transfer of oxygen from the air in the lungs to the various tissues of the body through the blood stream. Probably in many of the oxidation changes going on in the plant and animal cell, iron acts as the catalyst which speeds up the reaction.

Although iron is abundant in the soil and is required by plants in such small quantities, nevertheless, it is not uncommon to see plants in a chlorotic condition which may indicate iron starvation. This may result from conditions being inimical to bacterial activity, consequently the iron is not available.

Iron Bacteria.—There are living in the waters of certain muds and soils, bacteria which possess the power of abstracting iron from the water and depositing it on the surface of their bodies. It is to such organisms that the term "iron bacteria" is applied. They are of especial interest to the student of biology for two reasons: (1) Most of the organisms in this class are slightly higher in the scale of evolutionary development than are the majority of bacteria. They have taken the first step forward in what may be termed "communal life." In a very primitive way there appears to be a division of labor within the cells. (2) They are intensely interesting because of their peculiar habit of depositing iron on their bodies. How and why this peculiar condition is brought about or possessed by these organisms is not fully understood. Then too, they are of special interest to the engineer, chemist, and geologist on account of their ability to multiply rapidly and deposit great masses of iron. The result of this action is seen in the great deposits of bog iron which have come down to us from earlier geological periods. Today they often make their presence known by the deposits of iron which they accumulate in water mains. This at times becomes a real nuisance.

Harder divides the iron bacteria into three principal groups: (1) Those which precipitate ferric hydroxide from solutions of ferrous bicarbonate, using the carbon dioxide set free and the available energy of the reaction for their life processes (autotrophic). (2) Those which do not require ferrous bicarbonate for their vital processes but cause the deposition of ferric hydroxide when either inorganic or organic iron salts are present (facultative autotrophic). (3) Those which attack iron salts of organic acids, using the organic acid radical as food and leaving ferric hydroxide, or basic salts that gradually change to ferric hydroxide (heterotrophic).

These micro-organisms belong to the group known as higher bacteria and differ from true bacteria in morphology and methods of reproduction. They occur as long threads which are usually composed of many individual cells and present a variety of forms such as single threads composed of cylindrical cells placed end to end and generally enclosed in a sheath, ribbon forms produced by the bending of the filaments in the middle and the twisting of the ends around each other like rope. They generally reproduce by the formation of conidia or swarm spores. They belong to the family Chlamydobacteriaceae. There are five genera:

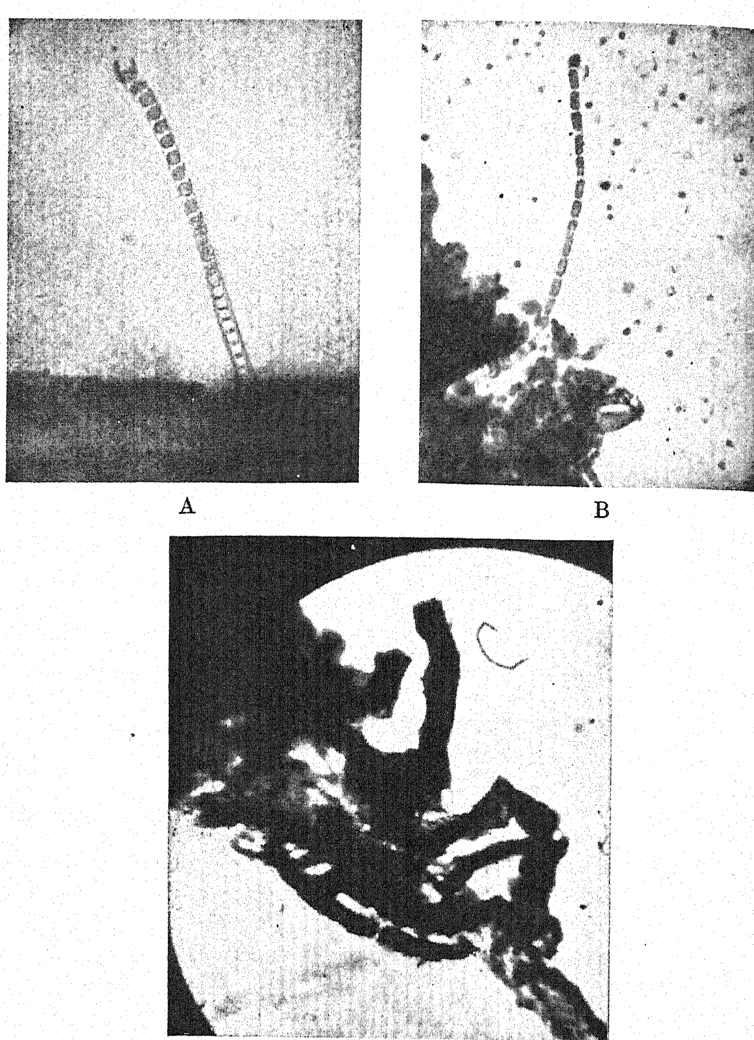


Fig. 73.—Iron bacteria (Crenothrix polyspora). *A,* From ferruginous water flowing into River Clyde. Condition slightly older than that depicted in *B.* Sheath is visible and open at the top. Cells in a single row; *B,* semi-mature thread. Thickness $^1/_{20}$ mm. Cells visible without treatment. Apex closed. Cells in a single row; *C,* basal parts of Crenothrix polyspora in state of decomposition. Described under the various names of Leptothrix meyeri and Megalothrix discophora. (*After Ellis.*)

Leptothrix, Didymohelix, Crenothrix, Sphaerotilus, and *Clonothrix.*

Opinions differ as to how they withdraw iron from solutions. Some workers have maintained that the deposition of iron hydroxide is a vital process of the organism and as such is carried out directly by the living cell. Others hold that the process is chemical and is incidental and not vital to bacterial life. Still others hold that the accumulation is mechanical and that particles of iron hydroxide in the water are caught and held mechanically by the mucilaginous parts of the bacteria. Probably different genera act in different ways.

Iron Bacteria as Pests.—Some iron bacteria are so common and are such a nuisance that in many places in the United States and Europe they are known as the "water-pest bacteria." They will attach themselves to stonework, woodwork, and pipes in reservoirs and water mains, and grow out into the water as fine threads. These continue to multiply until there results huge streaming masses of slimy material in which iron hydroxide is deposited. When the bacterial body disappears, there results a deposit of iron which, if on the inside of the water main, may result in clogging. They are, therefore, objectionable for a number of reasons: (1) They increase the amount of organic matter in the water, which in turn causes a great increase in the number of saprophytic bacteria in the water. (2) They decrease the bore of the conduit pipes, and cause a decrease in the velocity of the flow of the water and in time may result in a complete clogging of the mains. It is not necessary that the water be heavily charged with iron to bring about this condition for they grow well and absorb iron from water having only one part of iron in a million parts of water. (3) They may discolor the water and increase the sediment.

Two methods are in use for combating the action of the iron bacteria in reservoirs and water mains: (1) The organisms require ammonium salts for their growth and thrive in the presence of organic matter; hence, the removal of their food from the water will cause them to disappear. This is done by oxidation. Green plants may be grown which liberate oxygen, and the nitrifying bacteria oxidize the ammonia to nitrates. (2) The iron bacteria grow best in acid media; consequently, making the waters alkaline often prevents the growth of the pests and also accelerates the growth of nitrifying bacteria.

Bacteria in Relation to Iron Deposits.—Both chemical and biological processes have played an important part in the deposi-

tion of iron. In some deposits, chemical processes have predominated; while in others, bacteria have played a major part. Iron bacteria must have flourished in the rich, peaty water of the past and taken from them the iron and deposited it within their sheath. Later decomposition destroyed their bodies and there resulted the iron deposits, many of which are being mined today.

Bacteria and the Transformation of Iron in Soil.—Iron has long been known to be an essential plant food. Although it is not a constituent of chlorophyll, yet in the absence of iron, plants soon take on a yellowish or blanched appearance known as chlorosis. Due to the general distribution of iron in soils it has generally been believed that there is sufficient in soils for normal plant production, yet chlorosis often occurs in the plant. This raises the question: If iron is present in soil in sufficient quantities, is it there in the proper form for normal plant growth? It occurs in soils in both the organic and the inorganic forms. All of the organic and much of the inorganic iron is unavailable to the growing plant. The organic iron must be liberated by decay bacteria. During the process acids are produced which in turn may render the inorganic iron soluble. Ferrous iron is quickly oxidized by chemical and biological processes of the soil. Chlorosis may, thus, be an indication of slow or improper bacterial activities in the soil.

Their action at times, even in soil, may be injurious. Ferric iron under anaerobic conditions may be changed to ferrous, and ferrous iron is regarded as more toxic to plant roots than is ferric. Moreover the precipitation, under certain conditions, of colloidal ferric hydrate, may lead to the cementing of the soil grains with the formation of an iron "hard pan."

Stimulation of Bacteria by Salts.—Small quantities of most poisons when administered to man act as a stimulant, and it is only when the dose becomes large that the poisonous action is perceptible. This appears to be a universal law, holding for man, animals, and plants including bacteria, for it is found that when small quantities of alkali are added to a soil the bacterial numbers and activity increase. However, the extent of this increase varies with different micro-organisms and also with the salt. Small quantities of the carbonates, chlorides, sulphates and nitrates of sodium, calcium, and magnesium greatly increase the beneficial bacterial activities of many soils. The increased bacterial activity may liberate more phosphorus and potassium for the plant. Calcium sulphate or gypsum is especially active in increasing nitrification, and increases proportionately the plants

growing on that soil. The dealer in gypsum often takes advantage of this fact in advertising his materials by writing the word PLASTER with a heavy application of the material in large letters on a cultivated field in view of the public road. In the larger greener growth of grain crops or grasses the word PLASTER can be read by the passerby. In this way the farmers are induced to use gypsum or land plaster. This, however, is usually a stimulant, and if the landowner continues to apply the material year after year where the word was first written, the

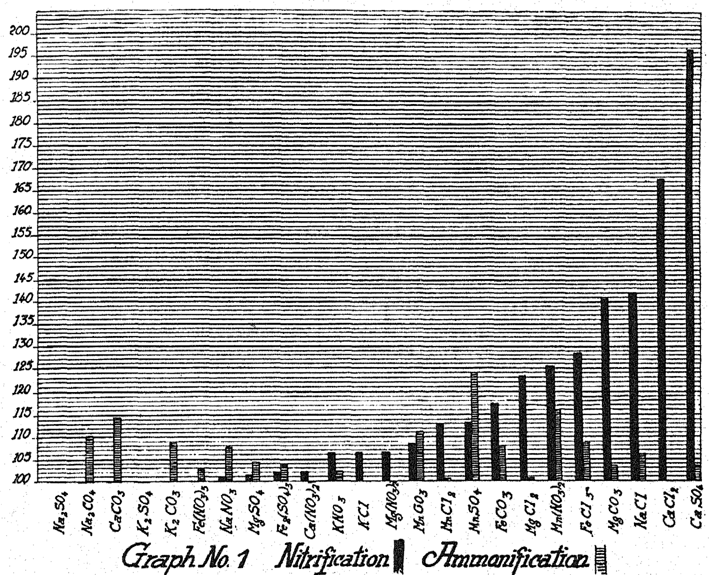


Fig. 74.—Illustrating the stimulation of bacteria by salts. The untreated soil is considered as 100 per cent.

time will come when it cannot be read. If he continues the application long enough, ultimately one may again read the word PLASTER, but this time the grain composing the word would be inferior to that surrounding it, and the physical properties of the soil, where the plaster has been applied, will be found to be bad.

Common salt is also often used as a plant stimulant. This mainly increases bacterial activity and with the exception of the gain in nitrogen it is also a stimulant. Now, it may be legitimate at times to use such substances, but the farmer should do so with the understanding that he is using a stimulant—not

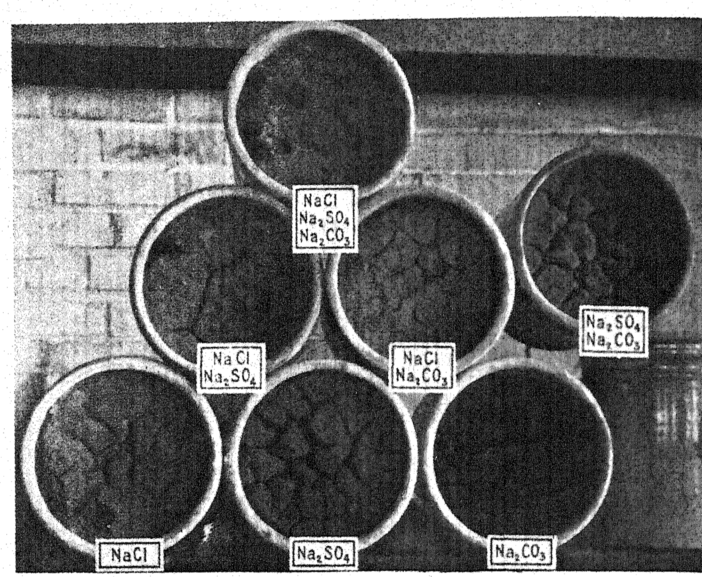


Fig. 75.—Appearance of alkali soil. (*From Greaves' "Bacteria in Relation to Soil Fertility." Published by D. Van Nostrand Co., New York.*)

a plant food—and that he is getting a temporary—not a permanent—effect.

Salt	Ammonifier (pounds per acre)	Nitrifier (pounds per acre)
Calcium sulphate..............	24,500	
Sodium carbonate..............	22,890	57
Sodium sulphate................	2,556	79
Magnesium sulphate...........	2,200	270
Magnesium chloride............	1,700	29
Sodium nitrate.................	1,530	47
Sodium chloride................	263	
Calcium chloride...............	61	313

Toxicity of Alkali Salts.—If we gradually increase the quantity of alkali salts in a soil, there comes a time when the bacterial activities are decreased. The quantity of alkali which must be added to a soil to make it poisonous to bacteria varies with the salt, the soil, and the bacteria. The quantity, stated as pounds per acre, which it was found necessary to apply to a calcareous

loam in order to become toxic to ammonifying and nitrifying organisms, is shown in Table on page 256.

These results are not to be taken as indicating that neither ammonia nor nitrate is produced in a soil containing the above quantities of alkali, but they simply indicate the concentrations at which the activities of the micro-organisms are slowed down in this particular soil.

Ammonia is produced in a soil containing considerable alkali, but the production of nitrates is interfered with even when small quantities of alkali are present. The great difference between the reaction of the ammonifiers and nitrifiers is due in part to the fact that many molds ammonify, whereas it is only bacteria which nitrify. Molds are considerably more resistant to alkali than bacteria.

Much larger quantities of alkali than those reported above have to be in a soil before the nitrogen-fixing bacteria are injured. In an alkali soil the nitrifying bacteria disappear first, followed by the ammonifying bacteria; and finally, in the barren waste the nitrogen-fixing micro-organisms disappear.

The first injury done to bacteria by alkali is due to an increased osmotic pressure of the soil solution, for the osmotic pressure of all these solutions at the point at which they become injurious is between 1.65 and 1.95 atmospheres. As larger quantities of the salts accumulate they poison the soil micro-organisms. Thus, alkali salts are highly fatal to the nitrifiers, less so to the ammonifiers, and still less to the nitrogen-fixing micro-organisms.

It is interesting to note that alkali salts are poisonous to nitrifying micro-organisms in much lower concentration than to higher plants. It is quite possible that the early injurious effect observed when land starts to "go bad" from an accumulation of alkali is caused by the action of the salts on the nitrifiers. It is, however, only after these salts become quite concentrated that they actually injure the plant. Moreover, after a soil has been leached of its alkali it is a number of years before it becomes productive. This unproductive period is in a measure due to the bad physical condition of the soil, but this is not the only factor, for the addition of manure or extracts of productive soil may greatly shorten the unproductive period by bringing to the soil the necessary microflora.

Variation in Toxicity.—The toxic or poisonous action of a salt, as already stated, varies not only with the kind and quantity of salt present but also with the soil. A quantity of common salt

or other alkali which would be sufficient to greatly retard bacterial growth in a coarse soil may be without effect in one of fine texture. Moreover, a salt is more poisonous in a soil low in organic content than in one of high. It appears that it is the greater surface exposed to the action of the salt in the fine-textured soil that holds the salt in such a way that the bacteria are not injured. This is spoken of as adsorption. Moreover, salts are less toxic where there is a mixture than where a single salt is present.

Antagonism.—If certain salt-water fish are placed in a solution of common salt having the same osmotic pressure as sea water, they soon die. Death in this case is not due to a lack of food, as similar fish placed in distilled water live for some time. If, however, a small quantity of calcium chloride is added to the first type of solution, the fish will live normally. Moreover, when the heart is removed from the body of a cold-blooded animal and placed in a salt solution it soon dies, but if a small quantity of calcium salt is present, the heart beats normally for some time. When one salt thus counteracts the poisonous effect of another it is spoken of as antagonism and a solution made up of a mixture of salts producing no toxic effects is known as a balanced solution

The property of antagonism also manifests itself in plants. One may take a soil which contains sufficient common salt to be injurious to plant life and by adding calcium salts to it overcome the toxicity. This appears to be a general law, for the injurious action of sodium carbonate, sodium sulphate, and a number of other salts toward bacteria can be neutralized by the use of calcium salts. The extent to which this neutralization can be carried is limited, as it is only the less concentrated salts which can be thus neutralized. However, this does indicate that a concentration of a single salt is often more injurious than is a similar concentration of several salts. We, therefore, have the peculiar phenomenon of being able to reclaim some alkali soils by adding more alkali to them.

Numerous workers have attempted to explain this peculiar phenomenon. Even before ideas on antagonism had taken a definite form, Löew elaborated a theory to account for the toxic properties of magnesium when calcium was not present in sufficient quantities. He supposed calcium to be a necessary constituent of the chlorophyll bodies and nucleus, and that when magnesium is present in great excess this takes the place in those bodies which should be occupied by calcium. This causes a structural disturbance on account of which the protein sub-

stances cease to be active and death ensues. It will be observed that this will only explain the need for a balance between calcium and magnesium and not between other compounds, as is often the case. Osterhout, who has done much work in this field, considers that the value of calcium lies in its effect at the surface between the absorbing membrane and the external solution and is not due to chemical action inside the cell. He, therefore, explains antagonism by assuming that antagonistic substances prevent each other from entering the cells, for, according to Loeb, if the wrong salts reach the inside of the cell they replace the salts in the protein and the cell dies. Out of this work has grown the idea of physiologically balanced solutions, or as Osterhout puts it, "normal life is possible only when necessary salts combine with the colloids of living substance in a definite ratio."

CHAPTER XXIV

WATER

NEXT to life, what is the most marvelous thing in the world? Is it the diamond, the hardest and most brilliant of all gems which is so prized by the young? Is it gold for which some will give even life itself? Is it radium, which is so rare that a ton of pitchblende must be worked over to obtain only 1 Gm.; which incessantly, and without appreciable loss, pours off rays that travel at the rate of 20,000 miles a second, forty thousand times faster than a rifle bullet; which penetrates thick blocks of metal, and which has such mysterious and wonderful action on living matter that it is being utilized in the curing of a number of ailments? No, as we shall see, it is common water.

Importance of Water.—Water composes two thirds of the human body. It enters into the makeup of every tissue. The muscles, which react so nicely to the will and perform such marvelous feats, contain 75 per cent water; the liver, which stands guard over the body, constantly protecting it from poisons, consists of 75 per cent water; the bones, which possess a tensile strength of 25,000 pounds per square inch and are one and one fourth times as strong as cast iron, consist of 40 per cent water; the brain, the most complicated and wonderful organ of the body, consists of 80 to 90 per cent water; the blood, that cosmopolitan fluid which visits every tissue of the body bearing to it nutrients and from it waste, contains over 90 per cent water. All the secretions of the various digestive glands consist mainly of water, which is present not merely as a vehicle in which the active principles are conveyed, but it actually enters into practically every change through which carbohydrates, fats, and proteins pass in the process of digestion and metabolism. Each 100 Gm. of fat burned in the body yields 107 Gm. of water. It is the fluid in which the mineral nutrients which play such a vital part in life phenomena are held. Water gives to the tissues plumpness, carries off waste, regulates body temperature, acts as a lubricant, and is a universal catalyzer.

The ancient philosophers recognized the importance of water, and Thales founded his philosophy and science on the idea that

water is the origin of all things. The elements of the ancients were earth, air, fire, and water. Mathews stresses its importance to man in the following words:

"It is marvelous to find that living matter with all its wonderful properties of growth, movement, memory, intelligence, devotion, suffering, and happiness should be composed to the extent of 70 to 90 per cent of nothing more complex nor mysterious than water. Such a fact as this is most perplexing, especially when all experiments show that the water is playing a profoundly important part in the vital phenomena. Any interference with the amount normally present makes a change at once in the activities of the cell. In fact, we might say that all living cells function in water. Not only is this obviously true in the lower and simpler forms of animals and plants, which are little more than masses of naked protoplasm living in the water, but it is also true of the higher forms in which the internal medium, or environment is of a liquid nature; the lymph, the blood, or the sap is the immediate environment of the cells. Water is the largest and one of the most important constituents of living matter, and if organisms are carefully examined the most various devices are found to assure the regulation of the water content of the cells of the body."

Animals whose habitat is far removed from the sources of water possess special mechanisms for their protection during water deprivation. The camel has not only a system of stomachs effectively arranged for carrying its water supply, but it has humps with great stores of fat which, when metabolized, yield large quantities of water. It also possesses a thick coat of hair which reduces evaporation to a minimum and a digestive tract which permits no waste of water. Man suffers little when dying from hunger as compared with thirst. At first, when deprived of water, the mouth and throat become parched, the saliva dry and sticky, the tongue clings to the teeth or the roof of the mouth, and there is a lump in the throat which causes endless swallowing. Later the eyelids stiffen, and the eyeballs assume a set sightless stare; delirium develops with visual illusions of lakes and running streams which are just beyond the reach of the individual, and in a short time the awful suffering is relieved by death. In starvation, an animal may lose practically all of its sugar and fat, half of its body proteins, approximately 40 per cent of its body weight, and still live. On the other hand, the loss of 10 per cent of the water content of the body results in serious disorders, and the loss of from 20 to 22 per cent results in death.

Nor do we have to confine our attention to biology when considering the importance of water. The physicist has chosen it to define his standards of density, of heat capacity, and as a means of obtaining fixed points on the thermometer. The chemist is often exclusively concerned with reactions which take place in water and which often are due to it. The geologist reads the history of the earth from the effect which has been produced by water. While controlled in the liquid, solid, or gaseous form, in the stream which turns the wheel of the electric generator, the ice which protects our food, or the steam which drives our engines, it is man's greatest friend; but, when uncontrolled in the raging torrent, or when congealing within or under a building, it becomes a relentless foe. Thus, considered from a physical, chemical, or biological viewpoint, water is the most important compound known to man.

Classification of Water.—Water may be classified as good, polluted, or infected. A good water, from a sanitary viewpoint, is one which will pass a satisfactory physical, chemical, and bacteriological analysis. This should also include a sanitary survey of the water shed.

A polluted water is one containing organic wastes of either animal or vegetable origin. It may or may not be a dangerous water, but it is a suspicious water.

An infected water is one which actually carries germs which cause human diseases. Such water carrying the germs of disease is dangerous, but it is seldom that bacteriological analysis can prove their presence. A drinkable water is sometimes referred to as a potable water.

Waters are very conveniently classified as atmospheric, surface, stored, and ground waters. The same water at different times plays different rôles; at one time it is in the atmosphere; later, on the surface of the earth; and still later, in the ground. In each case it is water, but from a sanitary viewpoint it has greatly changed. In the atmosphere, or just as it condenses, it may be nearly pure, but on reaching the earth it may become polluted, or even infected.

Atmospheric water may fall as rain, snow, hail or dew, and as such represents the purest natural water at our command. It is water which has been distilled by nature on a gigantic scale. As it falls through the atmosphere it becomes charged with dust, soot, bacteria, nitrous, nitric, and sulphurous acids, the quantity and kind of pollution collected varying with the locality and the time which has elapsed since the atmosphere was last washed.

On reaching the surface of the earth it is a safe water, although not free from foreign substances. Here, however, it quickly takes up more dirt, more organic matter, and more bacteria, some of which may be pathogens.

It is a well-known fact that some of the filthiest, if not the most dangerous water used for domestic purposes, comes from rain-water tanks. This is due both to the methods of collection and storage which pollute, but usually do not infect it.

Rain water is especially free from calcium and magnesium salts, hence is referred to as a soft water. It is especially valuable for cooking and laundering purposes. When the calcium and magnesium exist as bicarbonates, heating breaks them down with the production of insoluble carbonates. For this reason it is known as temporary hard water. Waters containing the chlorides and sulphates of calcium and magnesium cannot be softened by boiling and are known as permanently hard waters.

Surface waters include rivers, creeks, small streams, lakes, ponds, and reservoirs. Surface streams vary widely in purity depending upon the nature of the catchment basin and the season of the year. Streams which drain mountains or sparsely settled districts may be safe. Those which drain lowlands, especially those inhabited, generally are polluted and may be infected. The too general practice of running sewage into a nearby stream makes the latter a constant menace to health. The impossibility of protecting such waters from pollution is forcing the sanitarian to the conclusion that in as far as possible such waters should be purified before they are used.

Lakes, large ponds, and reservoirs, when fresh and kept free from pollution with human and industrial waste make admirable sources of water. They may be more easily protected than are streams and the natural agencies for purification—time, sedimentation, sunlight and enormous dilution—play a great part in freeing the water from any accidental pollution which may find its way into the water.

Ground waters are of two classes:

(*a*) Deep springs and artesian wells, from which most bacteria and suspensoids have been removed by filtration. Such waters in passing through the soil may take up large quantities of carbon dioxide. They have a great solvent action for lime and other inorganic constituents; hence, they are usually safe but hard.

(*b*) Shallow springs and wells correspond more nearly to surface waters and often are polluted and at times infected. Shallow wells and surface springs, especially if in porous soil, may

be polluted from toilets, or cesspools. The water from these seeps through the soil and carries with it the death-dealing microbe. Where the wastes are some distance from the well, especially where the soil is tight, the danger from this source is small as water may be purified by passing through a few feet of good, fine soil. In many cases, the little culprit journeys along the surface and into the water—not by its own volition, however, for these organisms are dependent upon man and the lower animals for their locomotion.

Fig. 76.—Improperly covered well, pollution can readily get in from the top.

Who has not seen the well, loosely covered with planks between which grasshoppers, toads, and manure may find their way? It is easy to understand how filth from the boots of working men, or from children playing on the cover, or from poultry walking about carrying infection on their feet may get into the water of such a well. One need not go far in many rural districts to find the outhouse freely open at the back so that fowls or pigs can walk under and from there onto the planks covering the well. The filth left on the planks is washed quickly into the

well. This would do no harm, if the disease germs were not present. There may be no known cases of typhoid or dysentery on the premises, but in every community the carrier, like the proverbial poor man, is always with us, and sooner or later his in-

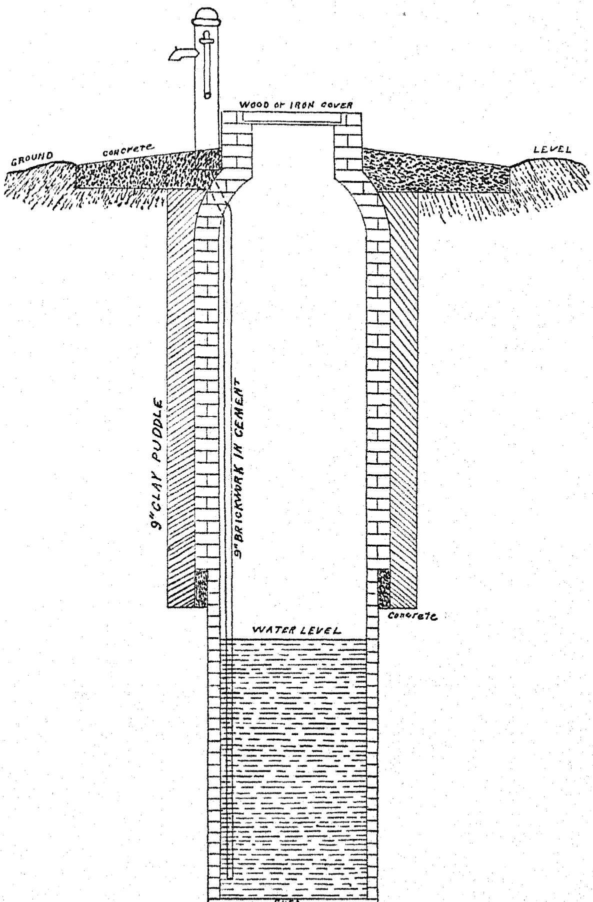


Fig. 77.—Properly constructed and covered well.

fection will be deposited in the outhouse and find its way to the well. The inhabitants learn of the presence of the disease germs when there is an outbreak of disease. The old lumber which covers so many wells should be replaced by tight-fitting cement platforms. These should cover not only the surface but several

feet of the soil surrounding the well and pass some 3 or 4 feet into the ground so that all the water which may find its way to the surface will have to pass through several feet of firm soil before it reaches the drinking water. It is probable that if this procedure were practiced generally, 90 per cent of the wells which are now a constant menace to health would yield safe water.

How to Tell Good Water.—The deep wells and springs usually are safe. But how are we going to know when we have the

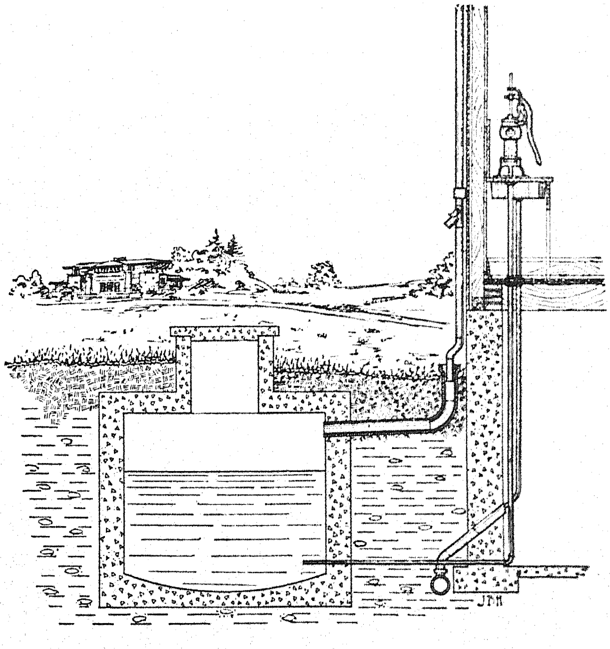


Fig. 78.—Properly constructed cistern located outside the house. (*Minn. State Board of Health.*)

deep spring or well? And how can we tell when pollution is finding its way into the water? Valuable information can be obtained on these questions by anyone who will apply a few simple tests.

In northern districts where there are great changes in temperature one often finds a spring, the users of which say, "The water of this spring is warm in winter and cold in summer." However, if the temperature of the water were taken with a thermometer during summer and winter, it is probable that it

would be found to be the same on both occasions. A uniform temperature indicates that the water is not coming directly from the surface and, therefore, is not likely to be polluted.

Even clear, sparkling water may be laden with death-dealing germs; hence, brilliancy and clearness do not insure purity. Yet that well or spring water which becomes turbid after a storm, if in the neighborhood of human habitation, is dangerous. The storm which carried into the water clay and mud and silt may also carry human filth, and this perchance may be the resting place of disease germs.

All ground waters when evaporated leave a residue. The nature of the residue varies with the water. Water free from pollution may give a voluminous white deposit when evaporated. When this deposit is dark it indicates organic matter. The odor of this dark mass when charred in the vessel in which evaporation occurs, may indicate the nature of pollution. That which comes from corrals and outhouses has an offensive, fetid odor which is not often found in the safe water.

Corrals, outhouses, and cesspools may at times be in the neighborhood of wells or springs, and one may wish to know if they drain into the well. A few simple tests usually will answer. Coal oil spread over the suspected area and then washed down with water or rain will appear in the well or spring water if there is drainage. This may be detected by the odor or the fine film which forms on the surface of the undisturbed water. Similar evidence may be obtained by the use of large quantities of salt placed in the suspected area and then tested for in the water.

More accurate information may be obtained by dissolving 1 Gm. of fluorescein in a little alkali, mixing with a quart of water, and flushing into the suspected place with large quantities of water. If there is a connection between the suspected polluted area and the well or spring, the fluorescein appears in the water to which it imparts a yellowish, fluorescent color so characteristic that one cannot mistake it. This test is very sensitive since 1 grain will impart a visible fluorescence to over 500 gallons of water. Moreover, if traces of it are left in the water after the test it is usually harmless.

From this it can be seen that the water of an unpolluted well or spring usually comes from deep in the earth, has the same temperature summer and winter, does not become turbid after a storm, and is not influenced by coal oil, fluorescein, or other substances placed in polluted areas.

Micro-organisms in Water.—Water receives its microflora from air, soil, and from the living and lifeless bodies of plants and animals. Consequently, almost any germ, pathogen, or saprophyte may occasionally be found in water. Many of these micro-organisms soon perish, some may survive for a longer period, whereas a few will find the water a suitable permanent habitat. It is the micro-organisms which make water their home that constitute the natural microflora of water.

The bacterial content of water varies greatly with the source, season of the year, and especially with the kind and quantity of pollution which it has received. Rain and snow water may vary from a few or even none to thousands per cubic centimeter, depending upon the nature of the atmosphere through which it has fallen and the surface on which it is caught. Deep springs and wells contain few or may be entirely free from bacteria. On the other hand, surface wells and springs contain thousands or even millions. Running streams usually contain more bacteria than standing water, but even in the case of these there is a wide variation, depending upon the turbidity and especially the sewage pollution which they have received.

Some sanitarians of wide experience are inclined to hold that natural waters which are found by approved methods to contain more than 1000 bacteria per cubic centimeter should be considered suspicious. However, numbers cannot be taken as the sole criterion of purity, as a turbid stream free from pollution may carry many times this number and yet could not be pronounced dangerous. The number of bacteria found in a water by the various methods must always be interpreted in the light of the chemical and further bacteriological analysis, and the sanitary survey. The specific value of the bacterial count rests on the information which it imparts on a water which has been studied over a period of time for a distinct increase in numbers indicates pollution. Then too, the bacterial count made before and after purification gives a fairly accurate measure of the effectiveness of the method used.

Inasmuch as counts do not give an exact measure of the purity of the water, and it has been found impracticable and usually impossible to isolate the pathogens from them, other methods have had to be devised to determine the purity of a water. The chemical methods determine the quantity and kind of nitrogen-carrying compounds in the water. From these determinations one may draw conclusions as to the nature, quantity, and quality of pollution which has entered the water, and what is

more important, the probable time which has elapsed since it entered.

The most valuable and widely used bacteriological procedure is to determine the presence or absence of *Bacterium coli* (*Escherichia coli*) in the water. This organism is an inhabitant of the human intestines, and, hence, is found in great numbers in sewage. Its presence in appreciable numbers is, therefore, presumptive evidence that sewage is entering the water and along with it pathogens may find their way.

Water and Disease.—Even as early as four hundred years before the beginning of the Christian era it was pointed out by some writers that there was a direct relationship between polluted waters and disease; some went so far as to advise the boiling or filtering of the water before it was used. In the centuries which followed, there was noted a direct relationship between drinking water and the great epidemics which swept over Europe. During the Dark Ages it was considered evident that water was often the cause of disease, but the real agent within the water was not suspected, for many a reputed witch lost her life for the alleged poisoning of water. The real cause was not determined until the nineteenth century, when Dr. Michel of France collected such an array of statistics that it seemed highly probable that typhoid fever was due in many cases at least to infected water. In 1880 Eberth discovered the typhoid bacillus, and in 1884 Gaffky succeeded in growing Eberth's bacillus upon laboratory media. Since that time evidence has slowly accumulated that definitely shows the causative organism of typhoid fever to be this bacillus. Furthermore, many typhoid epidemics have been traced to water, and in a few cases the causative organism has been obtained from the water.

Dysentery.—The relationship between impure water and disease is shown in the declining death rate from dysentery in the United States. The mortality from this cause in 1850 was 6.32 per 100,000 inhabitants; in 1860 it was 2.65; in 1870 it was 1.6; while in 1880 it was less than 1.5. During all this time the population had rapidly increased, and what is more important, it had been crowded together in great centers, thus giving greater opportunity for infection. There has been, however, much more attention paid to the water supplies of cities, resulting in a decrease in the death rate from intestinal diseases.

Typhoid.—The value of pure water is shown more clearly in another way. If one takes a five-year average of the deaths from typhoid fever in large cities both before and after the

installation of plants for the purification of drinking water, one obtains the results given in tabular form below. They are stated as yearly deaths from typhoid fever per 10,000 inhabitants:

Place	5 years before purification	5 years after purification
Hamburg	47	7
Zürich	76	10
Laurence, Mass	121	26
Albany, N. Y	104	28

This shows an enormous decrease in the death rate from typhoid on the installation of purification plants. It does not, however, tell the whole story, for eminent authorities on this subject have found that where one death from typhoid is prevented by improving the water supply, two or three deaths from other causes are prevented. An improved water supply not only reduces the number of deaths from typhoid fever but decreases infant mortality and the death rate from gastro-intestinal disturbances in general. This is due not only to the removal of disease-producing organisms from water, but also to the removal of many other substances and organisms in impure water which may greatly reduce the bodily vigor of the individual using it. Any thing, or any condition which in any way reduces the bodily vitality may make the individual an easy mark for disease.

Estimates vary as to the actual percentage of typhoid cases which are referable to water infection. It is placed by various authorities at from 10 to 40 per cent of the total cases. One of the reasons for this uncertainty comes from the fact that actual proof that a typhoid epidemic is due to infected water is usually indirect, for the positive isolating of the offending organism is effected with considerable difficulty and has been accomplished in only a few cases. However, strong presumptive evidence is obtained whenever waters are proved, through the presence of *Bact. coli,* to have been infected by sewage.

However, the best possible evidence showing that a specific typhoid outbreak is due to infected water is that obtained by the epidemiologist. He has learned that water-borne typhoid has certain important characteristics:

1. They may be preceded by a period of dysentery.
2. Water-borne epidemics usually have a sharp onset. The curve rises to a peak and then rapidly declines.
3. The cases are quite evenly distributed over the city; that is, provided the city is served by a municipal supply.
4. Water outbreaks nearly always occur in the spring, fall or winter.
5. The pollution is usually nearby and the epidemic is of short duration unless there is a continuous source of new infecting material.

Other Diseases Due to Water.—In the past, cholera has often resulted from the drinking of water containing cholera organisms. Even today this is true in some parts of the Old World. There is a marked difference between typhoid and cholera in that typhoid bacilli probably never multiply in water and rather quickly disappear, whereas cholera organisms will survive longer and may even multiply in polluted water.

Due to the fact that water is universally used to dispose of human excreta, it often receives tubercle bacilli and other disease-producing germs. These, too, may give rise to disease when they find their way into drinking water.

It is often stated that simple goiter is due to water; for individuals drinking certain waters develop it, whereas those drinking other waters may not. In the past, this has been attributed to hardness or organic constituents in the water, but today it is known that the lack of iodine in the water and food causes simple goiter and that when sufficient iodine is supplied simple goiter disappears.

Natural Purification of Water.—Nature is constantly conducting on a gigantic scale the purification of water. This is done by distillation, sedimentation, and oxidation including the action of light and filtration.

Distillation.—It is estimated that annually over 86,000 cubic miles of water are evaporated from the surface of the earth. This rises as a vapor and leaves the impurities behind; it condenses and falls to the earth in the form of rain, snow, or hail, nearly free from organic and inorganic matter and totally free from pathogens.

Sedimentation and Oxidation.—Surface waters which have become polluted tend to purify themselves. This is due to physical, chemical, and biological processes which are constantly at work. Suspended particles having a specific gravity greater than water tend to settle to the bottom. These carry micro-organisms with them. Sedimentation is small in running streams but very great in standing water. It is probably of first importance in lakes,

ponds, and reservoirs, and is the principal reason why these waters contain fewer bacteria than running streams.

Oxidation and reduction of organic and inorganic constituents of the water with the production of simple substances which are not well suited to the maintenance of life and growth of many forms of bacteria are constantly occurring. They probably play a greater part in running than in standing water. Sunlight which plays an important but unmeasurable part in freeing waters from micro-organisms is probably more effective in standing than in running water.

Furthermore, there are certain biological factors constantly at work which tend to free water of its organic material and its bacterial content. Many organisms find water an unsuited medium for their growth and die. Still others may find within the water certain factors which actively reduce their numbers. These conditions which are not well understood, are known as antibiosis or symbiosis with probably, in some places, the bacteriophage playing a predominating rôle.

Filtration.—Surface waters pass into the earth to reappear in wells and springs. In loose, gravelly, and limestone formation, this may be ineffective, whereas under appropriate conditions water passing through a few feet of fine grained soil may have its bacterial content reduced from 95 to 99 per cent.

Whipple states: "Sandy soil is a good filtering material, and when a well in such a soil stands at the center of a circle 25 or 50 feet in radius in which there are no privies, cesspools, sink wastes, or other sources of contamination, the water can usually be depended upon as fit for domestic use, provided of course, that the top of the well is properly guarded against surface wash." This will depend upon the nature of the soil through which the polluted water must pass. The United States Public Health Service reports the recovery of *Bacterium coli* at distances varying from 1 to 232 feet from an experimental trench made in sandy soil and containing excreta. The organisms were found to travel only in one direction—that of the ground water flow—consequently the importance of locating wells above outhouses and cesspools.

Artificial Purification.—Those methods which are so effective in the purification of water under natural conditions are the methods usually made use of in purifying it artificially. Only a few of the best known artificial methods can be briefly considered here.

The slow sand filter removes impurities by sedimentation, fil-

tration, and the biological destruction of organic matter and bacteria. It has been used extensively for over one hundred years, the greatest impetus being given to this measure in 1893 when Koch showed that the proper filtration of the water from the Elbe River saved Altona from the epidemic of cholera that devastated Hamburg which was using unfiltered water.

The sand filtration method consists in passing water through a layer of sand of such fineness and thickness that the requisite removal of suspended substances is accomplished. The filter as usually constructed is a basin having a water-tight concrete base

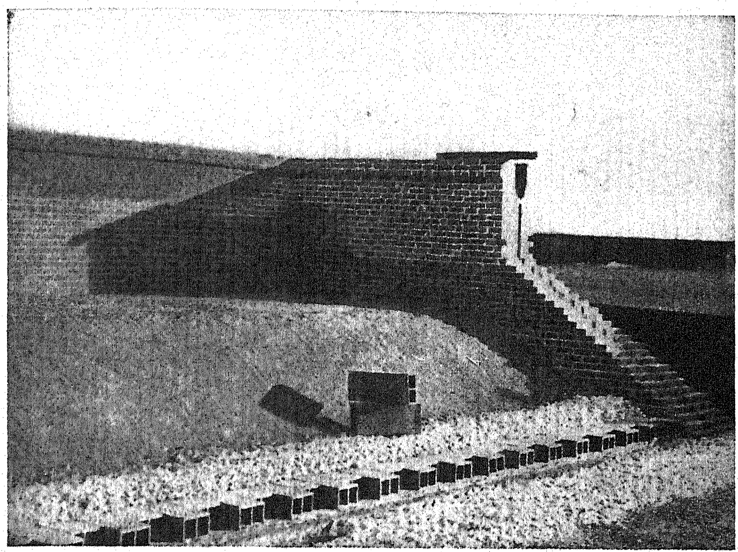


Fig. 79.—Cross-section of a typical slow sand filter. (*From Mason's Water Supply, John Wiley and Sons.*)

on the surface of which are laid perforated tiles or pipes. These tiles are covered with about a foot of gravel graded in size from 25 to 3 mm. in diameter from bottom to top. Over this are placed 3 or 4 feet of sand which acts as the real filter. The water passes through this and is conveyed to the mains by the underlying pipes. The suspended material, including bacteria, is removed by the sand which becomes more efficient as used, due to the rapid formation of a mat of fine sediment, in which protozoa often multiply, and assist biologically in removing many bacteria. In time the mat becomes very thick and filtration, although effec-

tive, is unduly slow. The water is then allowed to subside below the surface and about half an inch of the sand removed, after which filtration is resumed. The sand removed is washed to free it from collected impurities and later is replaced on the bed after successive scrapings have reduced the filter to about 1 foot in thickness.

The filters are usually divided into units of convenient size, about half an acre, so that one unit may be cleaned without interruption of the system. The slow sand filter removes about 99 per cent of the bacteria, about one third of the coloring matter,

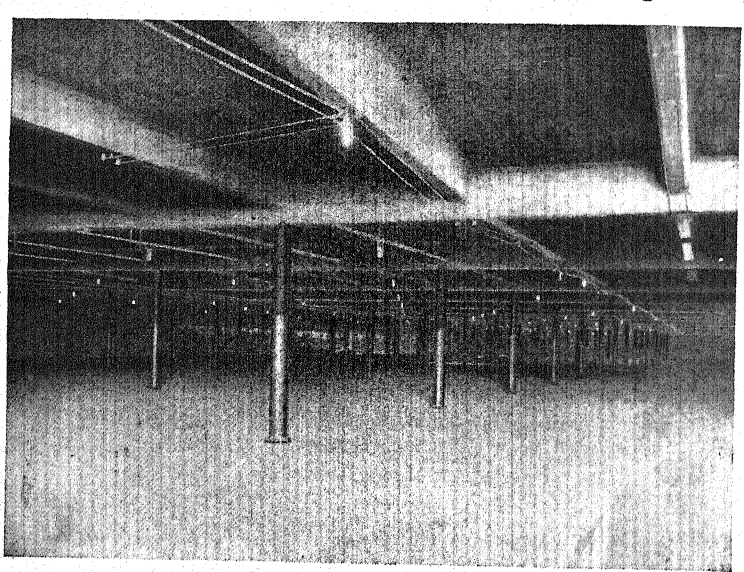


Fig. 80.—Filter bed No. 6 Indianapolis Water Company. (*From Boyd's Preventive Medicine.*)

and its long effective use has established the fact that it has a favorable effect upon the health of the community in which it is used.

Chemical Methods.—The chemical disinfection of water on a large scale is now almost exclusively effected with substances yielding chlorine, chief of which are bleaching powder (chlorinated lime), sodium hypochlorite, and free chlorine. The action of these substances is essentially similar and depends upon the quantity of active chlorine which they contain. They are usually added in quantities sufficient to give from 0.5 to 1 part of active chlorine per million parts of water.

The use of bleaching powder in the purification of waters is cheap, reliable, harmless, and easy of application, which makes it an attractive method, but when used on impure waters containing organic matter it gives rise to amines, chloramines and other compounds of unknown composition which impart to the water unpleasant flavors. However, it should be remembered that the treatment of water with chlorine to kill pathogens is useless in so far as amebiasis is concerned for it has been proved that the concentration of chlorine necessary to kill the cysts of Entameba histolytica is nearly one hundred times that used in water purification. Hence boiling or filtering affords the only practical means of rendering water safe which has been contaminated with the cysts of this parasite.

Alum is often used either alone or in connection with the mechanical sand filter, and if used under controlled conditions is very effective and leaves no undesirable constituents in the water. The quantity should be accurately determined for each water, for the effectiveness of it varies with the turbidity and quantity of calcium carbonate contained in the water.

Potassium permanganate is often used in disinfecting small quantities of waters, but its effectiveness cannot be depended upon except against the cholera spirillum. Moreover, the disagreeable taste and the color imparted to the water are a serious drawback. Ozone and ultraviolet light are both very effective in the sterilizing of water, but expense and technical difficulties have so far prevented their general use.

Boiling can always be depended upon for the rendering of a polluted water safe and may be used temporarily to tide over a short dangerous period. The difficulties involved in this method, and the tasteless water which results from it, are great drawbacks to its more general use.

The common filters which are on the market are usually unreliable, for if fine enough to hold back the impurities their action is slow and there is a tendency for them to leak. Furthermore, they may yield a bacteria-free water at first, but later the micro-organisms find their way through the filter. Hence, they can be depended upon only when properly constructed and cared for by experienced hands.

The Sanitary Drinking Fountain.—Drinking water should reach the consumer in an unpolluted condition. It is generally understood that the common drinking cup is a menace to health and in most cases this has been replaced in public places by the drinking fountain. However, there appears to be a widespread

lack of appreciation of the sanitary significance of certain features in the designing of a drinking fountain as improperly constructed ones are to be found in schools, railroad stations, and other public places. It has been amply proved that such drinking fountains can transmit respiratory diseases and that the vertical jet fountain with an exposed nozzle is only little better than the common drinking cup. With these facts in mind a committee of the American Waterworks Association has made the following recommendations:

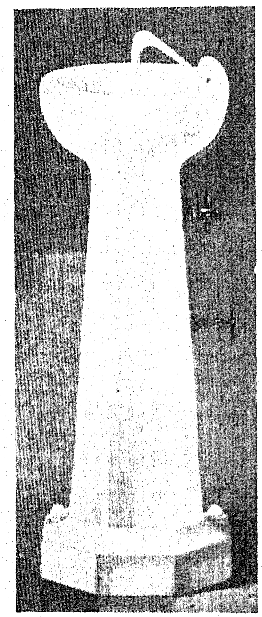


Fig. 81.—A sanitary drinking fountain. (*Courtesy of Crane Co.*)

1. All types of drinking fountains with vertical jets are to be condemned.

2. Most types of drinking fountains with slanting jets are to be condemned.

3. To be sanitary, drinking fountains should conform to the following specifications:

(*a*) The jets shall be slanting.

(*b*) The orifices of the jets shall be protected in such a manner that they cannot be touched by fingers or lips, or be contaminated by droppings from the mouth, or by splashings from basins beneath the orifices.

(*c*) The guards of the orifices shall be so made that infectious material from the mouth cannot be deposited upon them.

(*d*) All fountains shall be so designated that their proper use is self-evident.

Careful consideration by the committee of the requirements in the designing, construction, and operation of drinking fountains whereby such structures may be in reality sanitary, indicates that the following details should be considered:

1. The fountain should be constructed of impervious material, such as vitreous china, porcelain, enameled cast iron, other metals, or stoneware.
2. The jet of the fountain should issue from a nozzle of nonoxidizing, impervious material set at an angle from the vertical, and at an elevation above the edge of the bowl, so that the end of the nozzle will not be flooded in case a drain from the bowl of the fountain becomes clogged.
3. The end of the nozzle should be protected by nonoxidizing guards to prevent the mouth or nose of persons using the fountain from coming into contact with the nozzle.
4. The inclined jet of water issuing from the nozzle should not touch the guard thereby causing splattering.
5. The bowl of the fountain should be so designed and proportioned as to be free from corners which would be difficult to clean or which would collect dirt.
6. The bowl should be so proportioned as to prevent unnecessary splashing at a point where the jet falls into the bowl. Self-cleansing anti-splash rims are recommended.
7. The drain from the fountain should be connected to a separate waste pipe.
8. The water supply pipe should be provided with an adjustable valve fitted with a loose key or an automatic valve permitting the regulation of the rate of flow of water to the fountain so that the valve manipulated by the users of the fountain will merely turn the water on or off.
9. The control valve should be operated preferably by knee or foot action to avoid possible hand contamination.
10. The height of the fountain at the drinking level should be such as to be most convenient to persons utilizing the fountain. The provision of several steplike elevations to the floor at fountains will permit children of various ages utilizing the fountain. Elevations may be difficult to provide, however, at fountains recessed in walls.
11. The rate of flow and the pressure should be such that the water will not splash over the bowl. It should be at a rate not less than ½ gallon per minute and at nozzle pressure not exceeding 5 pounds per square inch.
12. The waste opening and pipe should be of sufficient size to carry off the water promptly. The opening should be provided with a strainer.

CHAPTER XXV

BACTERIOLOGY OF SEWAGE

The teachings of Pettenkofer during the latter half of the nineteenth century directed the attention of health workers to sewage as a cause of disease. He taught that fecal matter undergoes a ripening process in the earth and, hence, becomes the cause of typhoid fever. He did not consider the causative agent to be alive, but thought that due to chemical reactions there resulted noxious substances. These he considered may be dissolved in the water or even disseminated in the air and give rise to epidemics. Due to his teaching, cities and municipalities started to provide for the adequate disposal of their sewage. Immediately, there resulted a decrease in the gastro-intestinal diseases. Pettenkofer's theory was wrong, but as we know today it removed the causative organisms of disease which breed primarily in the body of man and leave in his excretions. Since then man has learned that for both esthetic and sanitary reasons, sewage must be properly handled.

Composition.—Sewage is a variable mixture. It has very aptly been defined as the water supply of a town or city after it has been used. It carries human and animal excreta, refuse from the kitchen, laundry, and manufacturing establishments. In cities having only a single system it may also carry huge quantities of dirt and refuse which come from the storms and flushing of the streets. Its quantity is directly proportional to the consumption of water in the districts. In small cities it may be as low as 40 or 50 gallons per capita daily, whereas in larger cities it may reach from 100 to 200 gallons or over.

The composition of sewage depends upon the density of population, the number and kinds of manufacturing establishments, and whether or not there is a separate or a combined system. Where the combined system is used, the composition and quantity of the sewage vary with the rainfall and the street washing. There is usually a diminution in quantity and change in composition at night. From the viewpoint of purification, sewage contains urea, various proteins (albumin, casein, fibrin), carbohydrates (sugars, starches and celluloses), fats, soaps, and other

organic constituents. The decomposition of the proteins yields ill-smelling, offensive nitrogen- and sulphur-carrying compounds. Fats are only slowly decomposed by bacteria and in the early stage give rise to glycerol and fatty acids. The fatty acids react with calcium and magnesium with the formation of insoluble soaps, which may collect on some solid moving substance such as a match, nail or other insoluble material. The water rolls them along, which often results in the formation of huge, insoluble balls that may become large enough to obstruct the flow of the sewage. Mixed in this heterogeneous mass are swarming hosts of micro-organisms most of which are harmless. They soon engage in the beneficent work of destroying the organic matter. Along with these may be bacteria from persons sick with typhoid fever, dysentery, cholera, tuberculosis, and other diseases; hence, "sewage is obnoxious to the senses because of its decomposing organic matter, but it is dangerous to health because of the possible presence of pathogenic bacteria."

Bacteria in Sewage.—The number and kind of bacteria in sewage vary widely and depend upon the composition and origin. Usually there are more present in European than in American sewage. There are also more in the day than in the night. However, this may be reversed in manufacturing centers where water from manufacturing plants finds its way into the sewers. The numbers of bacteria range from 500,000 to 12,000,000 per cubic centimeter. Fuller estimates that ordinary sewage contains about 322 billion per day for each person connected with the sewer.

Sewage may or may not contain pathogens, but millions of saprophytes are at all times present. Of the latter there are both anaerobes and aerobes. The common anaerobes are *Cl. regularis*, *Salmonella enteritidis* and *Cl. butyricum*. The aerobes are the common organisms from water, soil, air, and the human and animal intestinal tract.

Interest centers more in the changes produced by the various micro-organisms found in sewage than in the specific organisms. Most of the bacteria are not only harmless, but of genuine importance in the economy of nature through the scavenger work which they accomplish. A few of them are dangerous because they may cause certain infectious diseases. Many of them play an important rôle in decomposing sewage, producing malodorous gaseous products that are associated with putrefactive nuisances. The modern tendency, therefore, is to classify sewage bacteria from a physiological standpoint. They may be classified in

relation to their oxygen requirements as aerobic and anaerobic, or with regard to their action on a substance as hydrolyzing, oxidizing, and reducing. From the standpoint of sanitation it must always be kept in mind that although the majority of sewage bacteria are harmless saprophytes, there is always the possibility of dangerous pathogens being present.

Hydrolytic Changes.—Most of the early changes which occur in sewage are hydrolytic; that is, the substance is caused to take up water, becomes unstable, and for some reason breaks into fragments. The complex insoluble compounds are broken into simpler soluble substances. This progresses rapidly under anaerobic conditions as a great variety of bacteria decompose proteins under anaerobic conditions. This process converts proteins by steps to proteoses, peptones, peptids, amino-acids, and finally to ammonia, carbon dioxide, methane, hydrogen, and so on. It corresponds in the main with ammonification; however, the changes in the septic tank partake more of putrefaction, whereas in soil decay predominates.

Cellulose fermentation, next to protein hydrolysis, is the most important work of bacteria in sewage purification. Paper, cotton fabric, wood, and other cellulose-containing substances are rapidly decomposed by various micro-organisms with the production of soluble substances—starches, sugars, acids, and finally carbon dioxide, methane, and hydrogen.

Probably fewer micro-organisms possess the power of saponifying fat than of liquefying proteins or hydrolyzing cellulose; and as a consequence, fat accumulates and tends to rise to the surface out of the sphere of bacterial action. At times it may accumulate around some solid and give rise to "grease balls" which cause clogging of pipes. The fat which is acted upon by bacteria is broken into fatty acids and glycerin. The fatty acids are quite resistant to further bacterial activity, whereas the glycerin is rapidly broken into simpler products.

Oxidation.—The complex microflora of the sewage must have energy. This they obtain in a great measure from the oxidation of the comparatively simple products yielded through the hydrolysis of the proteins, carbohydrates, and fats. These are caused to take up oxygen with the production of acids and finally carbon dioxide and water.

The ammonia liberated through the de-aminization of the amino-acids is oxidized by the nitrosomonas to nitrous acid and by the nitrobacter to nitric acid.

Reduction.—The nitrites and nitrates produced by the nitri-

fying bacteria are in a great measure reduced to free nitrogen through denitrification. The sulphur in the protein molecule is liberated in the form of sulphates, sulphur dioxide, and hydrogen sulphide. The sulphates formed are reduced to hydrogen sulphide. This reacts with the small amounts of iron and other metals present and forms the black residue of metallic sulphides that are always found on the bottoms of tanks and streams in which sewage is decomposing.

All of these processes go on simultaneously in sewage, for one is dependent upon the other, there being a true biological cycle, as is pointed out by Whipple: "The decomposition and oxidation of the organic matter in sewage are brought about by bacteria, and the bacteria serve as food for protozoa and other forms of microscopic animal life. The dissolved organic matter in sewage serves as food for algae. These algae and protozoa are, in turn, consumed by rotifera and crustacea, while the latter form the basis of food supply for various aquatic animals and fishes. Thus, there is a continuous biological cycle. Again, animal forms require oxygen and produce carbonic acid, while plants consume carbonic acid and produce oxygen. Where these processes occur normally and with a proper equilibrium maintained between animal and plant life, offensive conditions do not result; but where abnormal conditions are produced, as, for example, by the discharge of excessive quantities of sewage or trade wastes into a stream, a depletion of the dissolved oxygen may follow, or there may be an overproduction of algae so that the conditions become offensive. It is coming to be realized that in order to properly determine the dilution required in any particular case the conditions required to bring about this condition of biological equilibrium must be determined."

Pathogens.—Owing to the origin of sewage, as before pointed out, it may contain pathogenic bacteria at any time. The common species found are typhoid, cholera, and dysentery; however, it is possible for any of the other disease producers also to find their way into sewage. This is especially true of tuberculosis, the causative organism of which is quite resistant to putrefaction and may survive for some time in sewage. Most pathogens do not multiply in sewage, and some, for instance the typhoid bacilli, seem to die more quickly in sewage than in fairly pure water. Jordan thus summarizes our present knowledge of the longevity of typhoid bacilli in sewage: "Laboratory experiments have shown that the typhoid bacillus can survive in sterile water in glass vessels for upward of three months, and for possibly two

or three weeks in unsterilized ground or surface water. Other evidence indicates that the bacillus is able to travel in water a distance of at least 140 km., and to retain its vitality in natural bodies of water for at least four or five days. It is possible that water may continue to be the vehicle of infection during a much longer period, but the available data point to a comparatively short duration of life of the specific germ in the water of flowing streams. Under ordinary conditions no multiplication of the typhoid bacillus takes place in water, even when a considerable amount of organic matter is present, but on the contrary, a steady decline in numbers goes on. The history of typhoid epidemics tends to show that sewage pollution is to be feared chiefly when the sewage is fresh, and that the danger of infection diminishes progressively with the lapse of time.

"In soil or especially the fecal matter of privy vaults the duration of life of the typhoid bacillus is much longer than in water. Levy and Kayser found typhoid bacilli in soil that had been manured fourteen days previously with the five-months old contents of a vault. The evidence that any genuine multiplication can take place in the soil is not convincing, but it has been proved that the bacilli may be carried by water currents to a considerable distance from the point where it was first introduced. Infection of wells and small water courses is thus brought about sometimes by the washing of bacilli out of soil in which they may have lain dormant for many months. The persistence of typhoid fever around certain habitations may be plausibly explained on the supposition of an extensive soil infection. There is no doubt that the practice of using human excrement for manuring vegetable gardens entails a danger no less real because often unrecognized."

Methods of Disposal.—The method selected for sewage disposal will vary with the district, location, and means at the disposal of the sanitary engineer. However, in all cases he must keep in mind convenience and public health. In rural districts the well-constructed cesspool may of necessity be used, but in the urban district it must not be tolerated. One of the readiest methods, and one which until the last few years has been generally used in this country, is to allow the sewage to flow without treatment into the nearest stream, lake, or harbor. This is very successful as long as the quantity is not excessive, the dilution great, and the receiving water not used by other communities for drinking and culinary purposes. Where this method is used the dilution should be great. The Chicago drainage canal

was designed on the basis of 3.3 cubic feet per second for 1000 people. The efficiency of purification, however, varies with the nature of the sewage. The presence of trade wastes, especially those of an oily nature, which float on the surface, may form scums which interfere with the absorption of oxygen from the air. Rapidly flowing streams, on account of their absorption of oxygen, tend to purify themselves more rapidly than do slower ones. Cold water holds more oxygen than warm water, and fresh water more than salt water; hence, there is a greater tendency for oxidation in cold fresh waters than in warm or salty waters.

There is, however, a growing demand that sewage be treated before it is thrown into streams or lakes. This may be done

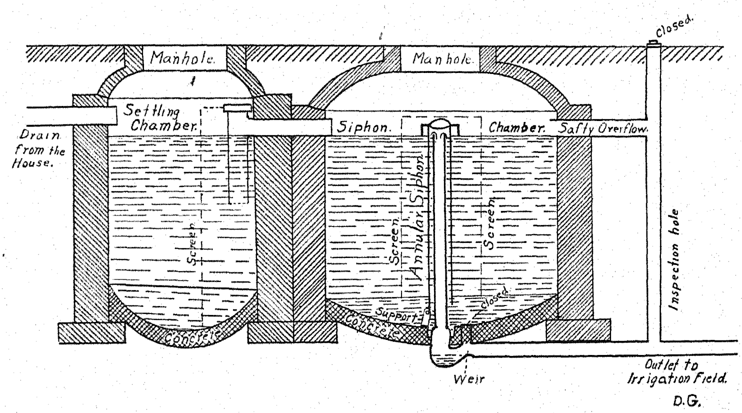


Fig. 82.—Settling chamber and fresh tank for surface and subsurface irrigation of sewage. (*Redrawn from Gerleard's "The Disposal of Household Wastes."*)

by various methods, such as sedimentation, subsurface irrigation, broad irrigation, and other means.

Methods of Treatment.—The methods used in the treatment of sewage depend upon the predominating changes occurring in the sewage and may be divided roughly into three classes: Physical, biological, and chemical.

The changes due to screening and sedimentation are primarily physical. When screens or strainers are used, they may be very simple metal rods or screens, or elaborate revolving cylinders covered with wire mesh. They are placed directly over the outlet of the sewer. In this manner the coarser materials which may interfere with later processes of purification are removed. In America these strainers are not used generally, for they re-

move only about 10 per cent of the suspended particles in American sewage.

Sedimentation is used much more extensively. This removes some 50 per cent of the suspended material, and depends on reducing the flow of the sewage which allows the coarser material to settle out. This may be accomplished by running into huge chambers where water currents are reduced to a minimum, or by filling a tank and then diverting it to a second tank while settling is occurring in the first. The coarse material which settles out is known as "sludge."

The sewage may empty directly or from the sedimentation tank into a septic tank in which it is retained for a considerable

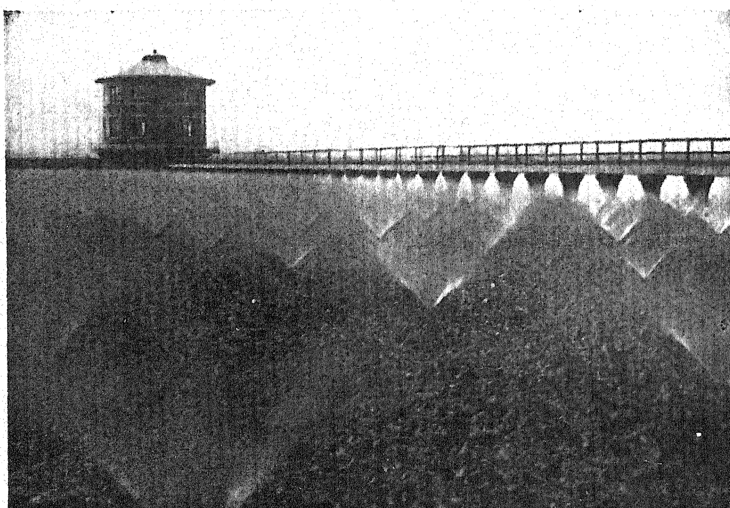


Fig. 83.—Trickling filters, Columbus, Ohio. (*Am. Mus. Nat. History Guide Leaflet No.* 33.)

time during which bacteria decompose much of the contents. During decomposition there results a film on the surface. This shuts out the air and favors rapid anaerobic decomposition. To prevent the gases from causing the mixing of this scum two story or Imhoff tanks are used. These are so constructed that the gases may leave and the sewage enter without disturbing the scum. The sewage from the septic tank may be conveyed to sand beds, trickling filters, sewage farms, or in other ways accelerate the biological processes so as to speed up aerobic decomposition and to completely oxidize the obnoxious sub-

stances which would otherwise remain. Often any one or a combination of the above processes is followed by a disinfection of the liquid portion of the sewage. This frees it of all pathogens. In the past chlorine has been quite generally used but within more recent years electrolysis has been found to have many advantages over simple chemical treatment.

Sludge.—In the sedimentation basins, in the septic and Imhoff tanks, in the contact and aeration bed, and after electrolytic and chemical treatment, there results a watery accumulation of organic matter, the disposal of which is of economic and sanitary

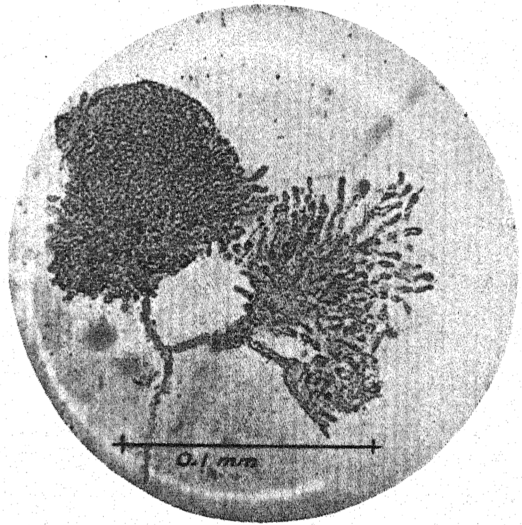


Fig. 83*a*.—Zoogleal mass from normal activated sludge floc, washed and teased free from nonzoogleal material (unstained). (*From C. F. Butterfield, Public Health Reports, vol.* 50, *No.* 20, *U. S. P. H. S.*)

interest. The bulk of this sludge consists of jelly-like masses, in which bacteria are present in large numbers. It is a typical zooglea formation, caused by the fusion of the gelatinous sheaths surrounding the bacterial cells. These zooglea are apparently the only growth in activated sludge which is a constant. Usually it is an irregular lobed mass, but at times is branching or filamentous.

For generations economists have recognized the enormous quantities of fat and fertilizer contained in and wasted in sewage. Hence, numerous attempts have been made to recover

part and in this manner reduce the cost of sewage disposal. Up to date this has met with only partial success. However, the two most promising methods are the activated sludge method and the Miles process.

In the activated sludge method raw sewage is run into aerating tanks in which air is pumped through the sewage by an air compressor. The air enters through perforated plates or pipes at the bottom of the tank. The sewage is then run into settling tanks and the sludge is collected. The resulting sludge is nearly free from odor and contains great numbers of saprophytic bacteria and is used as a fertilizer.

In the Miles process the organic material is precipitated and the fats hydrolyzed by means of sulphuric acid or in some cases, sulphur dioxide. The process removes from 61 to 66 per cent of the total suspended material and 99 per cent of the bacteria. The fats must then be separated and the products dewatered and dried. Up to the present time these last steps have not been economically successful.

What Should Be Accomplished in Sewage Disposal.—The sanitary engineer attempts to dispose of sewage as rapidly as possible, with the least nuisance to the smallest number of people, with the least damage to health or property, and at the smallest cost. Sewage can be made entirely harmless only by the complete destruction of its organic matter and bacteria. A complete purification is not attempted normally as the plant required for such would be very elaborate and too expensive. Moreover, practical experience has shown this to be unnecessary. What should be accomplished is simply a destruction of the disease-producing bacteria, the elimination of offensive odors, the destruction of solid organic matter, and the disposal of the liquid portion in such a way as to best aid natural factors in purification.

CHAPTER XXVI

MILK

MILK plays a double rôle in human welfare; that of a Dr. Jekyll and a Mr. Hyde. It is man's best food. It is all but indispensable to the growing child. It is the only food for which there is no effective substitute. It is also an excellent culture medium for bacteria, consequently, easily spoils. Due to its origin, method of handling, and nature, it probably causes more sickness and death than all other foods combined. Consequently, the principle underlying its production and consumption should be: Increase the production and consumption of milk, but see that it is good, clean, and safe.

Consumption of Milk.—The total milk production in the United States in 1932 was approximately 13,500,000,000 gallons. This would be sufficient to make a lake over 3 miles square and 20 feet deep. About 40 per cent of this was used as milk, and the remainder in the manufacture of butter and cheese. The average daily per capita consumption was thus slightly greater than 1 pint. Denmark, Norway, Sweden, and Germany all use over 1 pint per capita daily, whereas China, Japan, and the United Kingdom fall considerably below. In the United States the largest quantities are used in the eastern and western parts, whereas the southern part falls considerably below the average. More milk should be used, as is pointed out by Emerson.

"Present information as to cost and value makes it quite clear that the entire community would save expense and serve their nutritional needs if as much as 1 quart of whole milk were used as food for each member of the population daily. We can go further and say that it is indispensable for steady growth and development of children at least until they reach school age that a quart of milk should enter their daily dietary. We can add a third summary of the situation to the effect that unless at least a pint of milk a day per person is used in a community a waste of income and serious nutritional errors are certain to develop and affect the vitality, sturdiness and capacity to resist disease of a large number of both children and adults. Let me add further that none of these statements imply that these are measures of

what each person in the community is supposed to drink daily in the form of whole fluid milk. The use of milk in all forms of foodstuffs, soups, sauces, puddings, ices, hot and cold drinks, etc., is included in the daily designated allowance.

"This estimate of advantageous use of milk does not include the milk from which the finished products, butter and cheese, are made, nor does it include the skim milk powders, or the condensed and evaporated milk preparations.

"The above statements of an optimum per capita use of milk of 1 quart per day for the entire population and a minimum safe allowance of 1 quart for each child under six years of age and not less than 1 pint, for the remainder of the population is not based upon any single social, economic, chemical, or physiological test, study, or experiment, but represents the sum of observation in many relief agencies, budgetary studies of families under nursing and dietarian care, taken together with the experience of physicians and of medical surveys in hospitals, and the exact records of camps, institutions for children and adults, etc."

Value of Milk.—When milk is evaluated from the viewpoint of cost, digestibility, and nutritive value, it leads all foods. If cost is considered from energy values alone, milk is found even at present prices to be one of the cheapest foods. It is usually stated that 1 quart of milk is about equal in food value to any one of the following:

2 pounds salt cod fish	$\frac{3}{4}$ pound lean round beef
3 pounds fresh fish	$\frac{1}{3}$ pound cheese
2 pounds chicken	2 pounds potatoes
4 pounds beets	6 pounds spinach
5 pounds turnips	7 pounds lettuce
$\frac{1}{6}$ pound butter	4 pounds cabbage
$\frac{1}{3}$ pound wheat flour	8 eggs

This comparison is based on the energy content of the food and is only one, and at times the less vital rôle played by a food. In so far as energy is concerned the animal may use fats, carbohydrates, and proteins from different sources and interchangeably with nearly equal efficiency. However, this is not the case where food is used as a builder of human tissue. The individual breaks the proteins into their specific amino-acids; these are sorted over and built into the tissue proteins; hence, the quantity of tissue that can be produced from a unit weight of protein is governed by the kind and quantity of amino-acids contained in the food protein. Judged from this standpoint, the proteins of

milk are of a good nutritive quality and are superior to the proteins of most other foods, because milk and the leafy vegetables are the only foods so constituted as to make good the deficiencies of cereal grains, legume seeds, tubers, roots, and muscle meats. For this reason, McCollum has suggested that these two classes of foods should be designated "Protective Foods" in order to give them the prominence they deserve.

The experience of recent years has taught us the importance of the little things in the nutrition and well-being of man. Limitless quantities of carbohydrates, fats, and proteins fail to keep the human thyroid functioning in the absence of minute quantities of iodine. Likewise, a balanced diet lacking in vitamins may fail to promote growth, or may even fail to maintain life. Milk contains all of the known vitamins but is not uniformly rich in all. The fats of milk are a most important source of vitamin A which is essential for growth, as well as a protection against certain eye troubles and infections. It is also rich in the pellagra-preventing vitamin G.

Milk contains appreciable quantities of vitamin D which is essential in the metabolism of calcium and without which the animal develops rickets. It also contains some vitamin B and C. The quantity of some of the vitamins in milk depends upon the food received by the cow and in the case of vitamin C the treatment of the milk. Pasteurization destroys some of this vitamin. However, this cannot be urged as an objection against pasteurization as even raw milk should never be depended upon as the sole source of the vitamins. This is true especially in the case of vitamins C and D.

Calcium exists to the extent of several pounds in the adult body. It not only enters into the structural frame work but is a vital constituent of the body fluids and tissues. The typical American dietary with a large abundance of cereal grains of various sorts, of potatoes and meats, of sugar and fats, with a sparse inclusion of green vegetables is deficient in calcium; consequently, it is not surprising to find Sherman stating that a deficiency in calcium is one of the most outstanding defects in the American diet. There is little doubt that this is one of the main causes of malnutrition among school children. Milk remains today as the most available source of calcium, and if it were regularly included in the diet a considerable number of the many cases of malnutrition would disappear.

Moreover, individuals who have lived to a good ripe old age have usually used milk in some of its forms. Several French

laborers whose diet consisted largely of milk lived to be one hundred ten years and over. There are also authentic records of a number of individuals in the Balkans, Persia, Arabia, and in the Caucasus mountains who have reached extreme old age, and whose diet was largely milk.

Scientists have long studied the habits of these centenarians and their diet was found to contain large quantities of sour milk. Metchnikoff attributed their long life to a specific bacterium taken into the body with the sour milk, the *Lactobacillus bulgaricus*. Few today seriously subscribe to the original theories of Metchnikoff, but it has been demonstrated that the growth in the intestinal canal of the normal *Lactobacillus acidophilus* may be increased so as to make it the predominating organism by the liberal inclusion of fresh or soured milk in the diet.

The rôle of milk in the promotion of health and longevity is no longer a matter of theory and speculation for exact experimental evidence has been obtained by Sherman of Columbia University proving that the introduction of an optimum quantity of whole milk into the diet of rats increases their life span 10 per cent. This applied to the people of the United States suggests the possibility of adding six years to the present expectancy of life by the use of an adequate diet. The liberal inclusion of milk in the diet would go far toward accomplishing this end.

Milk in one form or another can be taken by most individuals. It is a very easily digested food, in fact it is often necessary to place children and individuals whose digestive systems have been impaired by faulty diet or disease on a milk diet. However, for the adult, milk is not a perfect food, because it is too bulky and is deficient in iron. That it has general far-reaching nutritive value is well understood. McCollum writes:

"Those people who have employed the leaf of the plant as their sole protective food are characterized by small stature, relatively short span of life, high infant mortality, and by contented adherence to the employment of the simple mechanical inventions of their forefathers. The people who have made liberal use of milk as a food have, in contrast, attained greater size, greater longevity, and have been much more successful in the rearing of their young. They have been more aggressive than the nonmilk-using people, and have achieved much greater advancement in literature, science, and art. They have developed in a higher degree educational and political systems which offer the greater opportunity for the individual to develop his powers. Such development has a physiologic basis, and there

seems every reason to believe that it is fundamentally related to nutrition." Yet surveys reveal the fact that milk, the best and most economical food, is used by the poorer classes in the smaller quantities, and what they do use is obtained in the most expensive form, condensed milk.

Classes of Milk.—It is generally recognized that there is a great difference in meat, bread, and vegetables, but it is not so generally understood that milk varies in quality and in nutritive value. All milk should be graded. Some writers say there are only two grades of milk—good and bad. There is a growing tendency to classify milk as pasteurized and raw milk. The United States Public Health Service in The Standard Milk Ordinance and Code classifies milk as "Grade A," "Grade B," "Grade C," and "Grade D."

"Grade A" milk is a good grade of milk obtained from healthy cows, by clean methods. It is produced under good sanitary conditions by individuals free from diseases. It is constantly kept clean, cold, and covered, and should not contain over 50,000 bacteria per cubic centimeter when delivered to the consumer. It should come from dairies which score at least 80 on the United States Bureau of Animal Industry Score Card. However, to make sure that it is safe it should be pasteurized.

"Grade B" milk comes from cows free from disease and is produced and handled under such sanitary conditions that the bacterial count never exceeds 200,000 per cubic centimeter. It should always be pasteurized under official supervision, and the bacterial content should not exceed 50,000 per cubic centimeter when delivered to the consumer.

"Grade C" milk is milk the average bacterial count of which at no time prior to delivery exceeds 1,000,000 per cubic centimeter. It conforms to all of the items of sanitation required of "Grade B" milk except that the cows need not pass the tuberculin test. Like "Grade B" milk it should never be used until pasteurized.

"Grade D" milk is milk which does not meet the requirements of "Grade C" milk. It is strictly an inferior milk and like "Grade C" should never be used until pasteurized.

In some places there is produced a certified milk which is the very best, freshest, cleanest, and purest raw milk that it is possible to produce. It is milk of uniform composition and of high quality obtained by cleanly methods, from healthy cows under special sanitary care. It is so certified by a medical milk commission. The cows have been examined by a veterinary sur-

geon to find if they are free from disease, especially tuberculosis. They are kept as clean as it is possible in a properly constructed, lighted, and ventilated barn. They are fed only good clean feed and water. A physician looks after the health of the milkers, and the greatest care is taken to see that the milkers wear clean suits, have clean hands, and use clean, sterilized milking utensils. The milk must meet certain high chemical and bacteriological standards. It must be bottled and iced at the dairy and should not be over thirty-six hours old when delivered to the consumer. It costs more than ordinary milk and is intended especially for babies and invalids.

Bacteria in Milk.—Milk is not only one of the best foods for man but it is also an excellent food for bacteria, as is seen from the fact that millions are often found in a few drops. In many cases the bacteriologist finds it one of the best media in which to grow his laboratory cultures. Milk should thus be produced and handled so as to keep bacteria as much as possible from entering. This is especially true concerning the pathogens. Large numbers of bacteria in milk indicate dirt, lack of refrigeration, or age. Such milk may or may not contain the germs of disease, but there is the possibility of it containing them. Hence, milk with a high bacterial content is not necessarily harmful, but when used as a food—particularly for children—is a hazard too great to be countenanced, or as stated by Conn: "Good, clean, fresh milk will have a low bacterial count, and a high bacterial count means dirt, age, disease, or temperature. A high bacterial count is, therefore, a danger signal and justifies the health officer in putting a source with a persistently high bacterial count with the class of unwholesome milk."

The number of bacteria occurring in milk varies with age, initial contamination, the care with which it is handled and kept, and temperature. Milk may contain a few or millions in each drop, or some market milks at times contain as many, but not as dangerous, micro-organisms as sewage.

The number of bacteria in milk is determined either directly or indirectly. Under appropriate conditions direct counts may be made of the bacterial content of milk or by a less direct method the numbers may be determined by plating on a suitable medium and later counting the number of colonies which have developed. Each has its advantages and disadvantages and gives only an approximation of the number actually occurring in milk.

The bacterial content and especially the keeping qualities of the milk can be quite accurately gauged by the methylene-blue

test. This is based on the fact that the color imparted to milk by the addition of the dye, methylene blue, disappears more or less quickly, the time required for its disappearance depending on a number of factors, the most important of which are the bacterial content of the milk and the temperature at which the test is made. If all controlling factors are kept constant except the bacterial content of the milk, the time required for the color to disappear varies with the number of bacteria.

Due to the inexactness of the information yielded by counts alone, some workers have searched for specific groups of bacteria in milk. The colon group has long been considered an important measure of sanitation as the members occur in the alimentary canal of animals. However, recent work makes it questionable whether great numbers of *Escherichia coli* are an accurate indication of sanitary milk production or simply age and improper refrigeration. They, like the actinomyces which get into milk from dust, hay, and straw, are objectionable because of their action on the milk and milk products. The presence of the colon group in milk used for making cheese gives rise to a product inferior in texture and flavor, whereas actinomyces may under certain conditions impart an obnoxious bitter-moldy taste to milk.

How Bacteria Get in Milk.—The bacteria that are present in the milk which reaches the consumer have come from two distinct sources: (1) The seeding of germs which the milk receives in its production and journey to the consumer; (2) the growth which takes place after the germs are seeded into the milk.

Milk as secreted by the healthy cow is a sterile fluid, but as drawn from the udder it is not. It may contain as few as 25 micro-organisms per cubic centimeter or the number may reach the thousands. Some cows give milk with few bacteria, others with many. Bacteria find their way through the orifice of the teat and, hence, are more numerous in the first milk than in the other milk. They are usually cocci and from the healthy cow are all nonpathogens.

During the milking process more germs are introduced. These come from (*a*) the coat, udder, and teats of the cow; (*b*) from the milking shed and clothes of the milker; and (*c*) from the hands of the milker. It is impossible to produce clean milk from cows that are so covered with filth that one cannot distinguish the color of their coats a few rods away. Even where the animal is in a fairly clean condition the wiping of the udder just before milking greatly reduces the number of bacteria in the milk. An

average of thirteen experiments at the Storrs Connecticut Experiment Station yielded the following results:

	Bacteria in milk per cc.
Unwiped udders	7048
Wiped udders	716
Decrease due to wiping	6332

Numerous investigators have shown the presence of large numbers of bacteria in cowsheds, and many individuals have seen stables or milk houses in which each beam of light passing through the crevices appears to be filled with myriads of dancing specks. These dust particles carry bacteria and if they find their way into the milk will increase its bacterial content.

Then, too, the hands of the milker may not be quite clean, and perchance have come in contact with disease germs from his own or someone else's body. These bacteria may find their way into the milk, where they sometimes multiply with enormous rapidity.

The extent to which the milker influences the bacterial content of milk is clearly illustrated by the following experiment reported by Stocking. The average of nineteen tests with two milkers, one with and one without previous training in dairy sanitation, showed 17,105 bacteria per cubic centimeter for the untrained man and 2455 for the trained man. The only difference was the knowledge possessed and used by the trained man.

Until a few years ago the barn and its surroundings were considered the main factors in governing the number of bacteria in milk. This feeling found expression quite frequently in summary orders from health departments to milk producers asking them to either provide better barns, or quit furnishing milk for the municipal supply. However, recent investigations have shown that in the matter of milk contamination the utensils used are of much greater importance than the surroundings. Many buckets are wrongly constructed or improperly cleaned; hence, every seam contains hidden millions of bacteria. These, on reaching the fresh, warm milk, immediately grow. Furthermore, the strainer may contain a goodly seeding of bacteria. It would, indeed, be a great step forward if the strainer could be done away with, for then greater care would have to be taken in the production of milk, in order to have the product fit to sell. The present condition is somewhat similar to that which existed

when it was first suggested that bread be wrapped. At a bakers' convention the subject of wrapping bread had come up for consideration and the members had practically agreed that all bread offered by them should be wrapped, when an old veteran arose and said: "If we wrap our bread in white paper and handle it as we do now the paper will be so dirty when it reaches the consumer that he will refuse to buy." So it is with milk; if it had to be sold in the condition in which it comes at times from the barn, it would be refused. Not that the strainer re-

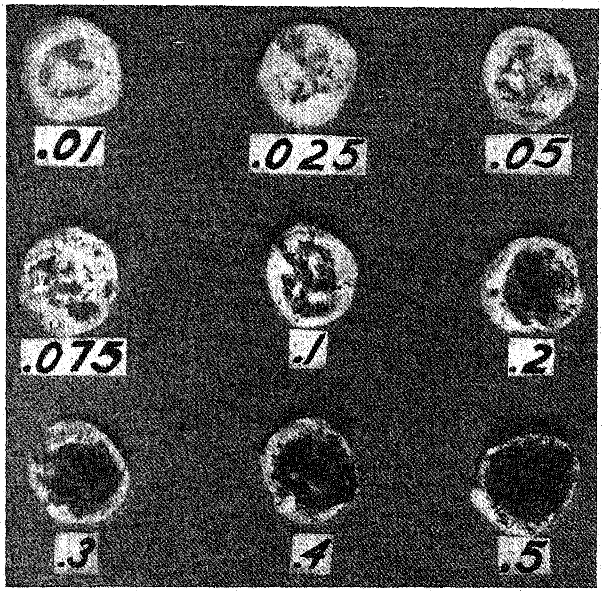


Fig. 84.—Sediment disks showing from 0.01 to 0.5 Gm. manure removed from different bottles of milk. (*Bull.* 642 *U. S. Dept. of Agr.*)

duces the number of bacteria in the milk, for it does not; it only removes the particles which are visible to the naked eye after they have been washed nearly free from bacteria.

Purcha and co-workers studied the influence of the utensils that normally come in contact with the milk both at the barn and at the dairy. They found that at least 80 per cent of the germ life that gets into milk comes ordinarily from the utensils in which the milk is handled. The milk can, the clarifier, and the bottle filler, when improperly cleaned and handled, were all prolific sources of contamination. Thus, while milk should be pro-

duced in clean, well-lighted barns, it is possible to produce a good grade of milk in poor barns; but it is impossible to produce good milk in improperly constructed and unclean utensils.

The contamination in transit and on the dealers' premises varies widely. Milk bottled and sealed at the dairy or municipal bottling plant can be delivered without any new seeding; but if it is pasteurized and then bottled or sold as "dip" milk from large containers, the additional seeding is often prolific. Milk should be sealed and pasteurized in the final container, and if the bottle is not covered with a cap fitting well over the neck of the bottle the top may be flamed for an instant before removing the cap. This will destroy any germs adhering to the neck of the bottle and is a worthwhile precaution, especially during epi-

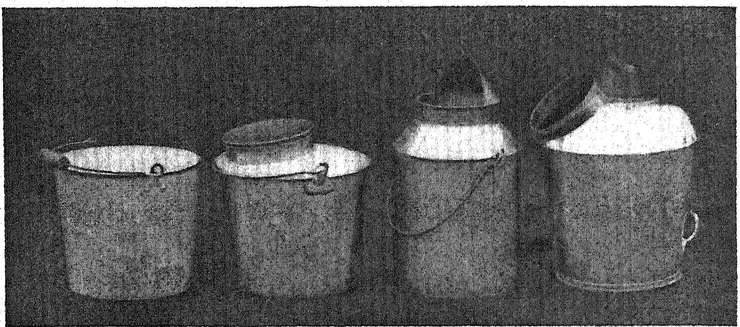


Fig. 85.—Types of milk pails. Narrow top pails are best. (*Bull.* 56 *Hygienic Laboratory.*)

demics, for it prevents the seeding of the milk as it is poured from the bottle.

How to Produce Clean Milk.—By clean milk is understood milk of good flavor, from healthy cows, free from dirt and containing only a small number of bacteria, none of which are harmful. The production of such milk depends upon (1) clean healthy cows, (2) the use of proper milk pails, (3) the handling by people free from disease, (4) the proper cleaning and sterilizing and drying of all utensils and containers which come in contact with the milk, (5) immediate and proper cooling, (6) storage at a low temperature, (7) keeping away from abnormal flavors and odors, and (8) prompt delivery.

Clean Healthy Cows.—The cows should appear well and show no udder trouble. The milk of cows suffering with garget should not be used. Cows should be tested yearly for tuberculosis, and

those that react should be removed and the remainder of the herd tested twice a year until the herd is entirely free. Cows should be kept in clean barns and pastures. They should be kept clean. The use of a brush and curry comb each day is often necessary to keep the cows in a clean condition. The clipping of the hair from the udder and flanks is a great aid, for this prevents dirt from sticking to them. A clean damp cloth should be used to remove dust and hair from the udder before milking.

Milk Pails.—Milk pails should be seamless and partly covered so as to prevent dirt from falling in from the body of the cows. The buckets should be biologically clean.

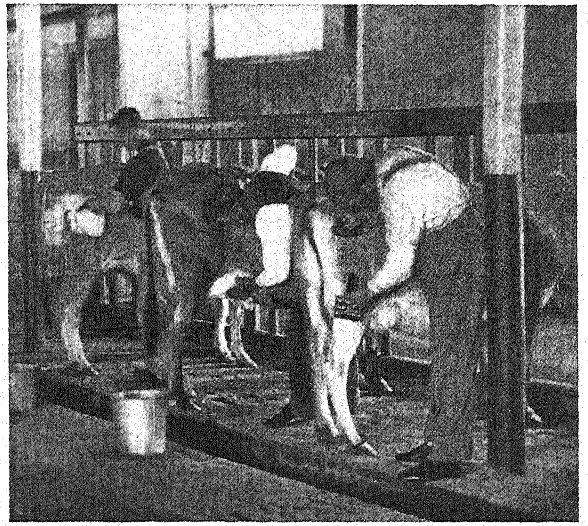


Fig. 86.—Cleaning cows preparatory to milking. (*Bull.* 56 *Hygienic Laboratory.*)

Milkers.—Milk should be handled only by healthy people. Individuals who are indisposed, suffering with sore throat, or who have been in contact with the sick should not handle milk as some of the pathogens, especially typhoid, readily multiply in milk and many of them may be carried in milk from the sick or healthy carriers to well individuals.

Clean and Sterilized Utensils.—Every container and utensil from the milking pail to the delivery bottle adds a few or many micro-organisms, depending upon its bacterial content. Recent work has shown that this is in many cases the most prolific source

of bacteria. Hence, milking utensils should be: (1) Properly cleaned with hot water; (2) sterilized with washing soda, live steam, or dry heat; (3) if soda be used this should be thoroughly rinsed from the bucket; and (4) except where dry heat has been used, the utensils should be carefully dried, which is done best with dry heat.

Cooling.—Milk should be quickly cooled to 50° F. or below. This can be done only by placing in cold running water or ice. The placing of large quantities of warm milk in a tub of cold

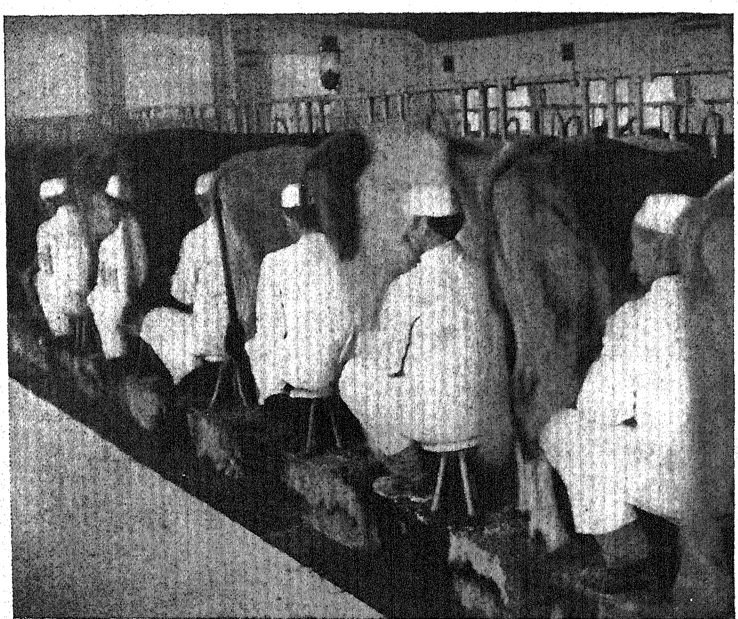


Fig. 87.—Clean surroundings, clean cows, milkers dressed in proper suits all help in the production of clean milk. (*Bull.* 56 *Hygienic Laboratory*.)

water is not sufficient to properly cool the milk. It may be stirred occasionally, so as to insure uniform cooling throughout but care must be exercised to see that dust and dirt are not added.

Storage and Delivery.—The milk should be stored at 50° F. or below and kept at this temperature during delivery. It should be delivered in closed vessels.

Growth of Bacteria in Milk.—Saprophytic and many pathogenic bacteria multiply in milk so that the number found in it is

governed, in addition to the factors considered above, by age and temperature. The influence of temperature is illustrated by the following:

Temperature maintained for 12 hours	Bacteria per c.c. at end of 12 hours	Hours to curdle at 70° F.
40	4,000	75
45	9,000	75
50	18,000	72
55	38,000	49
60	453,000	43
70	8,800,000	32
80	55,300,000	28

All of these samples at first contained the same number of bacteria but were kept for twelve hours at the different temperatures and then all maintained at the high temperatures. At the end of twelve hours there were over ten thousand times as

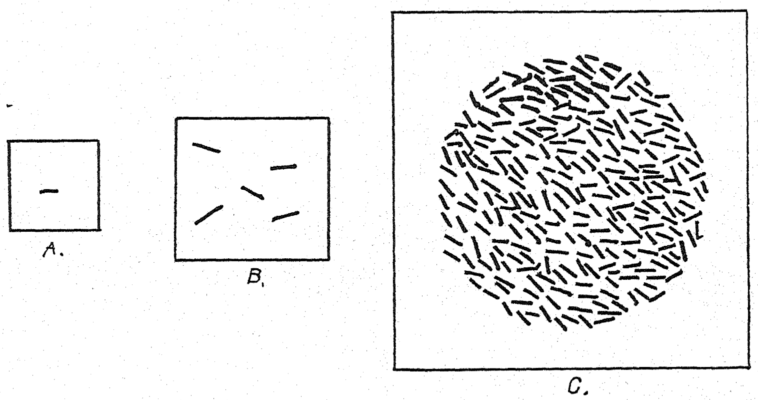


Fig. 88.—Diagram showing the effect of temperature on the rapidity of bacterial growth in milk. *A*, Original germ; *B*, growth in twenty-four hours when milk is properly cooled to 50° F. or below; *C*, growth in twenty-four hours when milk is not properly cooled.

many bacteria in the sample kept at a high temperature as in the one kept at a low. Although the difference in temperature was maintained for only twelve hours, the milk at 40° F. kept three times as long as did that held at 80° F.

Where milk is not properly iced during delivery there may be,

and usually is, an increase of over 500 per cent in the germ content above what it was when it left the pasteurizing plant.

Classes of Bacteria in Milk.—Milk obtains its microflora from various sources; hence, as is to be expected, it carries a heterogeneous lot which have been variously classified by different workers. Hasting roughly divides micro-organisms found in milk into five great classes: (1) Acid-forming bacteria; (2) peptonizing bacteria; (3) bacteria producing milk of unusual color; (4) inert micro-organisms, and (5) pathogenic bacteria.

Ayers and Johnson have grouped the saprophytic bacteria found in milk into five groups:

1. Acid coagulating—those bacteria which produce sufficient acid to coagulate the casein promptly.
2. Acid noncoagulating—those which produce acid, but do not coagulate the milk.
3. Inert bacteria—those which produce no visible change in litmus milk in two weeks.
4. Alkali-forming bacteria—those which produce an alkaline reaction in milk.
5. Peptonizing bacteria—those which liquefy the proteins of milk.

Normal Changes Produced in Milk by Bacteria.—Fresh normal milk may be slightly acid or slightly alkaline. When titrated with phenolphthalein as the indicator, it has an acidity which varies from 0.10 to 0.21 per cent expressed in terms of lactic acid. The acidity increases on standing, the rapidity depending upon the bacterial content and the temperature at which it is held. The increase is very slow at first. This is associated with the so-called "bactericidal phase," during which numerous germs which have found their way into the milk are destroyed, or at least agglutinated. Milk having an acidity of 0.3 per cent curdles on boiling and tastes sour to most people. Many pronounce milk sour when it has an acidity of 0.25 per cent. A few individuals will detect by taste an acidity of 0.2 per cent. When the acidity reaches from 0.50 to 0.65 per cent, varying with the sample, the casein is precipitated. The production of acid may continue until the acidity of the milk has reached 1.25 per cent. On reaching the maximum acidity, which varies with the temperature at which the milk is held, the acidity gradually decreases. After a time the milk becomes neutral and eventually alkaline.

During this series of changes first one type of bacteria and then another predominates, there being a sort of rotation of

minute plants in the milk. At times milk curdles and is still sweet; this comes from the growth within it of rennin-producing micro-organisms that attack the casein. The hay bacillus, as well as some of the micrococci may give rise to this condition.

Lactic Acid and Bacteria.—The bacteria which produce lactic acid in milk have been variously classified by different authors. Jensen divided them into three groups: (1) Those that grow at 25° to 50° C. To this group belong the long bacilli. (2) Those that grow at a wide range of temperature; namely, from 5–7° C. to 45–50° C. These are all streptococci. (3) The genuine lactic

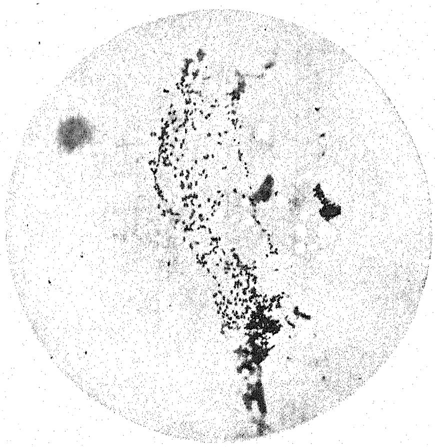


Fig. 89.—Microscopical appearance of milk that has been handled in poorly cleaned milk cans. This picture shows a film of short rod-shaped bacteria washed from the moist surface of the metal. Originally the film probably was much larger than this but was broken into pieces when the can was filled with milk. Even so, it probably would not break up completely into its component parts in preparing dilutions for agar plates. The species of this type of bacteria cannot be recognized by microscopical examination alone. (× 600.) (*After Breed.*)

acid bacteria that grow well at a temperature ranging from 10° to 40° C. This group does not include the coli-aerogenes group.

Löhnis proposes a more elaborate classification. He divides lactic acid producers into five groups according to their efficiency in producing lactic acid.

"Generally, the smallest quantities of acid are produced by the micrococci; next in activity come the intestinal lactic acid bacteria; stronger and much purer is the lactic acid formation by the streptococci; and the largest quantities are usually produced

by the lactobacilli, although a comparatively long time is required before the maximum is reached. The usual maximum for the streptococci is from 0.5 to 0.8 per cent lactic acid and for lactobacilli from 1 to 2 and sometimes 3 per cent. Naturally, this general classification does not fit every individual case; exceptionally strong and weak varieties may be found in every group.

"Whether one or the other group predominates in milk or in dairy products depends on the mode of infection, the temperature, the presence of air, and the composition of the substrate.

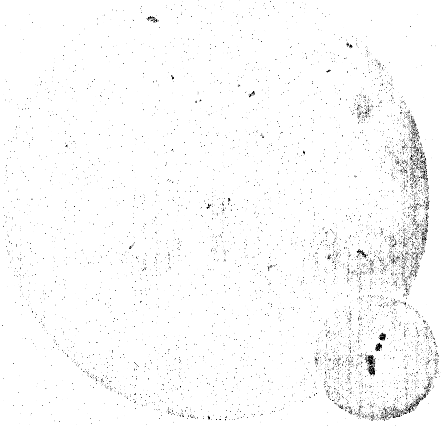


Fig. 90.—Microscopical appearance of milk, sixteen hours old, souring normally. The predominant organism is *Streptococcus lactis Löhnis*. This occurs normally in milk as pairs, threes, or double pairs of lance-shaped cocci. Milk of this type is usually produced through improper cooling, and it is usually at least twelve to sixteen hours old when examined. Less commonly, the same condition is produced immediately on filling a can that has previously contained sour skim milk or similar material. (× 600.)

The small insert shows two pairs of *Streptococcus lactis* (× 2000) taken from this preparation. One pair shows faint indication of division into four cells. (*After Breed.*)

"Concerning the mode of infection, it is to be pointed out that the micrococci come mostly from the udder, from the air, and from the utensils; the intestinal lactic acid bacteria, from feces, from impure water, and from unclean vessels; the streptococci mostly from containers, more rarely from the cow (udder, hair, etc.); the lactobacilli from silage and other food, saliva, feces, soil, and cheese. If the temperature is kept below 10° C. micro-

cocci will predominate after a few days; around 20° C. streptococci are very active; 30° to 40° C. are most suitable for the majority of the intestinal lactic acid bacteria; and at 45° to 50° C. hardly anything but lactobacilli survive. This general classification is again not without exceptions, but it is well worth knowing that around 20° and 45° C., the cleanest and most rapid formation of lactic acid bacteria is to be expected, while below 10° C., and between 30° and 40° C., many by-products are formed which act unfavorably upon taste and flavor. If milk cultures of lactic acid bacteria are kept continually at high temperatures, practically all of them are killed after a few days, while at 0° C. most cultures will remain alive for a long time, especially if enough chalk is added to neutralize the acid formed. The presence of air favors micrococci and intestinal bacteria, while the majority of streptococci and lactobacilli grow best under anaerobic conditions."

Abnormal Changes in Milk.—At times foreign undesirable micro-organisms find their way into milk and produce abnormal, objectionable changes.

The *Bact. coli* and the *Bact. aerogenes* types produce considerable gas and disagreeable odors and flavors in milk and dairy products.

Bacterium viscosum and related species produce a slimy or ropy condition in the milk. The slimy condition is thought to be caused by the mucin containing capsule which surrounds these bacteria. This change in milk has been known in every land and suggests that the causative organism is quite generally distributed. According to Hammer and Prucha these organisms are kept down by the normal souring of milk; but when the acid germs are destroyed there is a greater tendency for these organisms to make their presence known. However, by exercising care in the production of milk and especially in the sterilizing of utensils, the trouble caused by these undesirable bacteria may be eliminated. Slimy milk occurs more often in fall and spring, when the producer, vender, and consumer consider it unnecessary to ice milk; hence, proper refrigeration helps greatly in the control of this nuisance.

Besides those species mentioned above, there are several other groups of bacteria that often impart bad flavors to the milk in which they grow. For example, the actinomyces under certain conditions of storage bring about an obnoxious bitter moldy taste in milk. These organisms are numerous on straw, hay, grain, and soil, and reach the milk from these sources. The bitter

flavor bacteria cause in milk may result from the hydrolysis of the proteins of the milk with the production of proteoses and peptones. Milk that putrefies with the production of a bitter alkaline product is likely to cause illness, if used. Many other off-odors and tastes, such as fruit-like odors, soapy milk, and the like, may occasionally occur in milk because of the presence of specific micro-organisms.

Numerous bacteria possess the power of producing pigments when grown in milk; which accounts for the colored milks that one finds occasionally. A yellowish color is due to certain micrococci or short rods, a greenish discoloration to *B. fluorescens.* Blue milk may be due to *Ps. cyanogenes,* a pink color to *Serratia marcescens,* a deep red to *B. erythrogenes,* and a yellow to *Ps.*

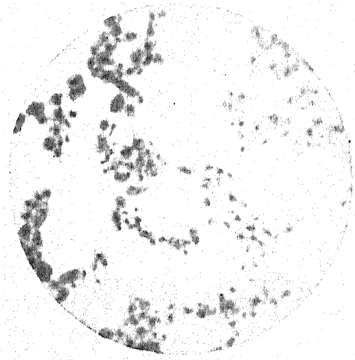


Fig. 91.—Bact. lactis mucosum. (*After Buchanan and Hammer.*)

synxanthus. Practically all of the organisms producing these various changes in milk can be considered nuisances, but they are not necessarily danger signals.

Milk and Disease.—As already mentioned, disease-producing micro-organisms often find their way into milk. The principal diseases conveyed by milk in the order of importance are: Tuberculosis, undulant fever, typhoid fever, septic sore throat, scarlet fever, and diphtheria. Occasionally other diseases such as foot-and-mouth disease, Malta fever, anthrax, and indefinite digestive disturbances may result from infected milk. Recent work has shown that undulant fever may result from the use of milk containing the organism which causes contagious abortion.

Typhoid fever, diphtheria, and probably scarlet fever come only from human cases or carriers. Septic sore throat and tuber-

culosis may be either of human or bovine origin, whereas the other named diseases come only from diseased animals.

Milk is infected in numerous ways, chief among which are the following:

1. The pathogens may find their way into the milk from diseased animals.

2. The milker or vender of the milk may be suffering with a communicable disease in a mild unrecognizable form.

3. A third and very important source of infection comes from carriers who work on farms, in dairies, and other places where milk is handled.

4. A rather common source of infection is where the milker or vender of milk comes in contact with sufferers of communicable diseases while attending to his regular dairy work.

5. Occasionally infected water is used for the washing of milk utensils, and in this manner the milk becomes infected.

During the six-year period from 1924 to 1929 there were officially reported in the United States 258 milk-borne epidemics. These epidemics involved 10,906 persons and resulted in 371 deaths. The relative importance of milk in the conveyance of various diseases may be seen from the following table given by Kelley and Webber for Massachusetts:

Disease	1907–1915		1915–1918		1919–1923	
	Cases reported	Per cent traced to milk	Cases reported	Per cent traced to milk	Cases reported	Per cent traced to milk
Typhoid fever	23,482	9.43	6,331	7.83	4,165	7.23
Septic sore throat	......	....	1,401	61.88	829	14.23
Scarlet fever	70,569	3.89	25,328	.55	46,777	.11
Diphtheria	69,646	0.18	33,807	.08	42,389	.018

In addition to this, many cases of tuberculosis are conveyed by milk. Rosenau estimated that approximately 7 per cent of the cases of tuberculosis in human beings are brought about by infected milk.

The principal reasons for milk-conveyed disease should be clearly understood. These have been summarized by Rosenau as follows: "(1) Bacteria grow well in milk; therefore, a very slight infection may produce wide-spread and serious results. (2)

Of all food-stuffs, milk is the most difficult one to obtain, handle, transport, and deliver in a clean, fresh, and satisfactory condition. (3) It is the most readily decomposable of all food. (4) Finally, milk is the only standard article of diet obtained from animal sources consumed in its raw state."

Pasteurization.—The fact that pathogens may find their way into milk during its production has led many health workers to hold that there is no more excuse for the drinking of raw milk than there is for eating raw meat. Probably the former is more dangerous than the latter; but be it as it may, the fact is that there is a growing demand for pasteurization which is variously defined by different state and city laws. Generally speaking, it simply consists of heating milk to a temperature below the boiling point and above 60° C. for a given length of time. The object of this treatment is twofold: (1) It destroys all pathogens which may occur in milk. (2) It increases the keeping qualities of the milk.

There are two general systems of pasteurization in common use:

1. Pasteurization with the application of a high temperature, 80° to 90° C., or sometimes higher, for a period of three minutes. This is known as the flash method.

2. Pasteurization at a comparatively low temperature, from 60° to 65° C. for from twenty to thirty minutes, and which is known as the holding method. Now that the main technical difficulties in this method are being overcome it is the one most generally preferred. Pasteurization is specifically defined in "The Standard Milk Ordinance and Code Recommended by the United States Public Health Service for Adoption by Cities" as follows:

"The terms 'pasteurization,' 'pasteurized,' and similar terms shall be taken to refer to the process of heating every particle of milk or milk products to a temperature of not less than 142° F., and holding at such temperature for not less than thirty minutes in pasteurization apparatus approved by the health officer, provided that approval shall be limited to such apparatus which requires a combined holder and indicating thermometer temperature tolerance of not more than 1½° F., as shown by official tests with suitable testing equipment, and provided that such apparatus shall be operated as directed by the health officer and so that the indicating thermometers and the recording thermometer charts both indicate a temperature of not less than 143½° F., continuously throughout the holding period, provided that noth-

ing contained in this definition shall be construed as disbarring any other process which has been demonstrated as of at least equal efficiency and is approved by the state health authority."

The milk while being pasteurized should be in closed vessels, preferably in the container in which it is to be delivered to the consumer.

A properly pasteurized milk is a safe milk in which all of the pathogens and approximately 99 per cent of the total bacteria have been destroyed. Pasteurized milk spoils as does raw milk, and if carelessly handled may be reinfected; hence, pasteurization is merely a preventive measure and should supplement and in no way replace the principles laid down for the production of a clean milk.

Evaporated Milk.—Evaporated milk consists of whole cows' milk concentrated to slightly less than one half its original volume. It is then canned and processed under steam pressure. The finished product should be sterile; however, this is not always the case and it may spoil. There have been cases reported in which the milk became bitter but otherwise appeared normal. The bitter flavor came as a result of cleavage of the proteins by specific micro-organisms.

The raw milk used in the production of evaporated milk should be of a high grade, low in acidity, and free from abnormal flavors. If the acidity is high, it coagulates on evaporating. If abnormal flavors are present they become intensified.

Sweetened Condensed Milk.—Sweetened condensed milk is concentrated whole milk to which approximately 40 per cent of cane sugar has been added. It is not sterilized, and consequently contains bacteria; but they seldom multiply in the high concentration of sugar, although some workers have found as many as a million micro-organisms in a cubic centimeters of sweetened condensed milk. Most of the bacteria present are micrococci, which give rise to a thickening of the milk. Various bacilli have also been isolated from sweetened condensed milk and some of these cause abnormal flavors.

Milk Powder.—This is prepared mainly by two processes. In one, the milk is dried in films on hot rollers; in the other, it is sprayed into hot chambers and dries while in the air. In this latter case, the proteins remain soluble. Milk powder has the advantage that it may be kept over long periods, as the finished product seldom has over 2.5 per cent moisture. The drying probably destroys all pathogens but not all saprophytes. The number of these in the powder as it leaves the drier is very low,

usually only a few hundred per gram. However, it may receive a liberal seeding in the handling processes. If stored for some time, all except the more resistant spores die. The remade milk powder is highly nutritive, acceptable in taste and appearance, and in most cases can be used wherever milk is used. It is low in vitamins, and when brought back into solution spoils just as does fresh milk.

CHAPTER XXVII

MILK PRODUCTS

Slightly over one half of the 13½ billion gallons of milk produced annually in the United States is used for the production of butter, cheese, and other milk products. The nature of these products varies with the milk from which they are made, the methods used in their production, and the chemical and biological changes which occur in the finished product. The changes occurring may be desirable or undesirable and depend upon the seeding of micro-organisms which the milk has received and the conditions under which the products were produced and stored. Modern bacteriological investigations make it possible to control the nature of the germs, and to a degree the changes which they produce, and hence, to control the nature of the finished product.

BUTTER

Composition.—Butter is prepared from cream, by churning, and is a mixture of fats, water, milk proteins, pigments, and other products. It varies in composition, as may be seen from the following summary of 695 analyses of American creamery butter reported by the associates of Rogers in "Fundamentals of Dairy Science."

Composition of American Creamery Butter (Per Cent) *

	Water.	Fat.	Salt.	Curd.
Maximum	16.83	86.91	5.26	3.42
Minimum	10.52	76.64	0.92	0.20
Average	13.90	82.41	2.51	1.18

* From "Fundamentals of Dairy Chemistry" by Associates of Lore A. Rogers, Reinhold Publishing Corporation, Publishers.

The fat is a mixture of olein, myristin, palmitin, stearin, laurin, butyrin, and, in smaller quantities, other neutral fats. Many of these fats on exposure to air and light, and especially under the influence of bacteria, yield glycerin and fatty acids. Butyric

acid is generally considered to be the cause of the rancid odor of old butter but the removal of the rancid odor by aeration does not materially reduce the free fatty acids. The unsaturated fats on storage may be oxidized, the rate depending upon (1) whether the cream has been pasteurized, (2) the temperature at which the finished product is kept, and (3) the presence of certain inorganic constituents. The salts of iron, copper, and nickel greatly accelerate the development of oily, fishy, and metallic flavors.

The moisture, lactose, and casein which are held mechanically within the butter are the pabulum in which the micro-organisms multiply. When the actions of the micro-organisms are inhibited by salt or low temperature, enzymes continue to act favorably or unfavorably on the texture and flavor of the butter. However, it must be remembered that the flavor of butter is a composite result developed from a number of factors. The milk, whether the cream was raw or pasteurized, the kind of starter used, method of production, age, and temperature at which the butter is stored, all play important rôles in determining flavor.

Micro-organisms in Butter.—The germ content of butter varies with a number of factors, the more important of which are:

1. *Cream.*—Butter made from sweet, clean cream has a low bacterial content at first, as compared with butter made from sour cream. Sweet cream butter lacks flavor and the yield is less than from sour cream. The micro-organisms in butter made from sour cream are principally lactic acid bacteria; however, it may contain many others, such as pathogens which have been obtained from butter made from infected milk. Market milk, due to its great variation, yields a variable product. In the more modern dairies the cream is first pasteurized and then seeded with a culture of lactic acid bacteria. This has advantages, in that it destroys all pathogens which may be in the cream and approximately 99 per cent of all bacteria in the cream. Hence, when inoculated with a reliable starter, ripening proceeds normally, and the product is of uniformly high grade. The temperature usually recommended for pasteurization is between 74° and 80° C. Temperatures below this yield a poor keeping product, while at higher temperatures the flavor is affected. The ripened cream contains from ½ to 3 billion bacteria per cubic centimeter. The number in the butter varies with age and conditions as well as with conditions under which it was produced and stored. A butter made from sweet cream appears to be better suited to the growth of bacteria than one from ripened

cream, and in most instances growth is more rapid at the surface than the interior. Ordinarily, conditions in the butter are unfavorable for the growth of bacteria. Few develop in the fat itself and the liquid phase contains from 14 to 15 per cent salt which is too high a concentration to permit the growth of most bacteria. A few salt-tolerant molds may grow, but these are minimized by proper wrapping and storage.

2. *Water.*—The water used in washing the utensils and rinsing the butter may carry into it many objectionable micro-organisms. Water used for these purposes should be low in bacteria and above suspicion; otherwise, it should be boiled before using.

3. *Salt.*—The salt may carry into the butter many undesirable resistant micro-organisms, and if it contains small quantities of iron or copper may give rise to unfavorable flavors in the butter.

4. *Utensils.*—The utensils used in the handling of milk are often responsible for 80 per cent of the micro-organisms added to it. Many of these are undesirable. The best churns and containers are made of wood. Metals tend to impart to butter offensive flavors. All apparatus must be carefully sterilized and dried each time after using.

5. *Paper.*—The paper used to wrap the butter may also carry undesirable germs or substances which impart to butter off-flavors. Molds are especially likely to be added in this manner. The previous boiling of the paper in a 25 per cent salt solution not only destroys the micro-organisms but removes from it any objectionable flavors. As all molds are aerobic, the exclusion of air by proper wrapping prevents their growth.

Kinds of Micro-organisms in Butter.—Many saprophytic and occasionally some pathogenic micro-organisms occur in butter. The principal pathogen coming from the cow is the tubercle bacillus which in the past has been occasionally found in butter. This can be entirely eliminated by the use of pasteurized milk, or milk from nonreacting tuberculin-tested cows. Typhoid bacilli may reach the milk from missed cases, the healthy carrier, or infected water. Typhoid bacilli readily multiply in milk but tend to perish in butter. However, this organism has been obtained from butter under experimental conditions several weeks after inoculation. The longevity of *Eberthella typhosa* in butter varies greatly with the temperature and composition of the butter, but the evidence is conclusive that butter should not be produced from infected milk. Pasteurization of the cream and the careful supervision of the workers is the only safeguard available in the prevention of butter infection.

The main saprophytic organisms in butter are the various lactic acid bacteria. The principal type is usually the streptococci, but the microflora varies with the organisms accidentally or intentionally seeded into the milk and cream and also the temperature at which it is stored. According to Löhnis, butter contains also quite regularly, though in small numbers, *B. fluorescens, Cl. butyricum, B. subtilis,* and *B. mesentericus.* Low-grade butter is comparatively rich in *Escherichia coli,* and also contains active actinomyces, yeasts, and mold. The molds multiply at the surface of improperly wrapped butter or in a product in which considerable air has been incorporated. It has been found that in dairies producing butter with a high yeast and mold count, lax methods of pasteurization and handling of cream prevails; hence, the yeast content is quite generally used as an index of efficiency.

Commercial Butter Cultures.—Some races of the human family, especially the Chinese and Japanese, prefer sweet cream butter, whereas the majority prefer sour cream butter. In order to obtain the latter product, the cream is allowed to sour. Until recently, and in many cases even today, the practice has been to allow the cream to sour spontaneously. Due to the great variation of the natural microflora, this process results in products varying in flavor which are often defective. When in 1890 Weigmann introduced into the dairy industry the process of artificially souring cream by the aid of pure cultures of selected races of lactic acid bacteria the process became controllable, and today the dairyman can produce day after day butter of the same flavor and aroma.

At one time it was believed that these butter cultures or starters, as they are sometimes called, contained only lactic acid bacteria, but today it is the prevailing opinion that in addition to *Streptococcus lactis* there are other "associated" organisms which Hammer divides into two types—*S. citrovorus* and *S. paracitrovorus.* The fundamental difference between them is that *S. paracitrovorus* produces some lactic acid in milk, while *S. citrovorus* does not. Both organisms produce volatile acid from lactic acid but the greatest amount is produced by *S. paracitrovorus.* Consequently, the "starters" as used at the present time are mixed cultures of organisms which have been obtained from milk and milk products and which experience has taught produces the desirable flavor and aroma in butter. They are used at the present time very extensively. Pasteurized cream can be used,

inoculated with the "starter" and permitted to ripen. This insures a uniform product.

Commercial butter cultures are supplied by various laboratories in either liquid or powdered form. Liquid cultures are usually propagated and supplied in milk whereas powdered cultures are prepared by taking good butter cultures, adding them to inert material such as starch, lactose, or milk powder, and then drying. The liquid cultures are active and quickly give a good starter but they have poor keeping qualities. The powdered cultures have good keeping qualities but are slow starters and if care is not taken in their preparation, foreign organisms are introduced with the inert material with which they are mixed.

CHEESE

Composition.—Cheese consists mainly of casein, fat, water, small quantities of other milk constituents, salt, and often added coloring material. The nature and composition varies widely with different kinds. There are about 400 varieties which can be grouped into some 20 distinct types, as many of the varieties differ only in name and most of the names originated in some locality where the cheese was manufactured. The different varieties depend upon the nature of the milk from which the cheese was made, the methods used in its manufacture, and the conditions under which the ripening process took place. The manufacturing process can be divided into four stages: (1) The casein is coagulated into a mass of curd by means of pepsin or rennin in the presence of lactic acid produced from the milk sugar by the bacteria originally in the milk or added with the enzyme. The coagulum that results from the action of the rennet holds most of the fat and some of the milk solids. (2) By special manipulation the curd is separated from the whey. (3) The curd is salted and compressed to give it definite form and to remove the whey. (4) The cheese is kept for a period ranging from a few weeks to several months under definite conditions of temperature and moisture, depending on the variety of cheese being made. During this ripening or curing process, various changes occur due to bacterial and enzymic activities which impart to the cheese its characteristic flavor and aroma.

Micro-organisms in Cheese.—It is desirable that the micro-organisms in milk and butter be low, but in cheese great numbers of the proper micro-organisms are essential to bring about the desirable changes. The number and kind vary with the milk from which the cheese is made and with the rennet used (home pre-

pared rennet is high in bacteria), the methods used in making the variety of cheese, and the age. About three fourths of the bacteria which occur in milk are carried into the curd of the hard rennet cheese. During the first few days there is a rapid multiplication of bacteria, which soon obliterates any differences in the milk; hence, the finished product depends upon the kind of bacteria. The maximum numbers are reached in from two to five days. There may be several billions in 1 Gm. of cheese. The microflora of young cheese may consist almost entirely of *Streptococcus lactis.* These are replaced as the lactose disappears, almost quantitatively by the lactobacilli. During the ripening, the casein is broken into simpler compounds including histidine, tyrosine, guanidine, and lysine. Still later there appear ammonia and esters, which probably give to cheese their characteristic flavors and aromas. After the preliminary lactic fermentation, in some cheese molds grow and impart their characteristic flavors. Molds grow on the surface, and ripening proceeds inward in Camembert, Brie, and similar cheeses, whereas in Roquefort, Gorgonzola, and Stilton they grow in the interior and ripening is uniform throughout.

A general idea of the microflora of the American cheddar cheese may be obtained from recent work at the New York Agricultural Experiment Station. Forty different samples of this cheese, all of which were obtained from different factories and from the open markets were studied, and no less than 265 cultures were isolated from them. These cultures were subjected to the usual laboratory tests and classified into the following groups:

1. *Spore Formers.*—Fifty-four cultures. From the better grades of cheese only 9 per cent were spore bearers.

2. *Gram-negative Rods.*—Fifty-two cultures. They were found in cheese of the poorer quality with gas and undesirable flavors.

3. *Lactobacilli.*—In the better cheese this group predominated and probably played the important rôle in its ripening.

4. *Streptococcus Lactis.*—In the early stages these organisms predominate and change the sugar to lactic acid.

5. *Cocci.*—Twenty-seven strains of cocci—20 white, 6 yellow, 1 orange.

6. *Streptococci Other Than S. Lactis.*—Twenty-six cultures, mainly from the poorer grades.

7. *Yeast.*—Three strains of yeast were obtained from the cheese and in one poor grade cheese the yeasts were the predominating organisms.

In the typical American cheddar cheese there is a rotation of germs during the ripening process. In the early stages the *S. lactis* type develops rapidly and may for a period be the predominating organism. In the better quality of cheese the *lactobacilli* and *cocci* develop up to the fourth or fifth month, after which the numbers of organisms diminish. In the poorer grades of cheddar cheese the spore formers and gram-negative rods develop after the first initial predominance of *S. lactis*.

Change in Ripening of Cheese.—Micro-organisms and enzymes are absolutely essential for the obtaining of desirable changes in the ripened cheese, for these organisms split carbohydrates, fats, and proteins and produce compounds which impart to cheese

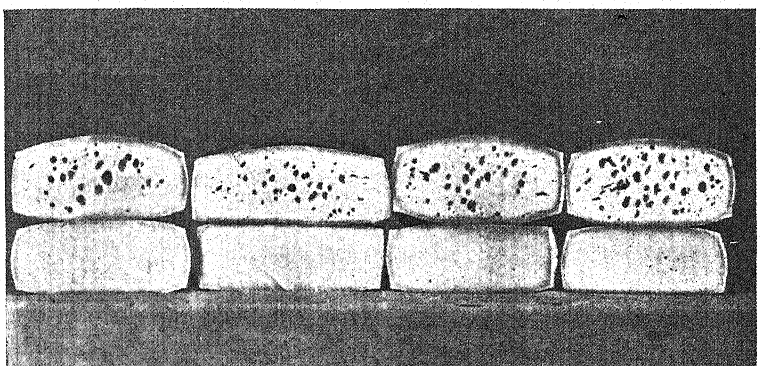


Fig. 92.—Influences of bacteria on cheese. The lower row of cheeses were made from milk lacking in the bacteria essential for the proper ripening of Swiss cheese. The cheeses of the upper row were made from the same milk as their respective "blind" controls, with the addition of a pure culture of the eye and flavor producing organism. (*After Sherman.*)

its specific texture, flavor, and aroma. During the early period of ripening, the milk sugar is transformed into lactic acid. This is indicated by an increase in the acid content of the cheese. The acid reacts with the casein and calcium compounds producing salts of lactic acid; which in turn are further acted upon by micro-organisms with the production of carbon dioxide, simpler acids, and small quantities of alcohol. Some of the casein, which is the principal protein of cheese, is broken down by the proteolytic ferments and soluble nitrogen-containing substances such as proteoses, peptones, amino-acids, and ammonia are formed. The nature and quantity of the final products vary with the kind of cheese, the time of ripening, and the temperature

at which it has been stored. The splitting of the fats varies even greater than the changes in the other constituents. Fats are split principally by aerobic germs; hence, this process proceeds most rapidly in soft cheese. If carried too far it may give rise to a rancid cheese. To retard the action of aerobes, cheese is usually covered with paraffin or other substances. This is especially effective in preventing mold growth.

Abnormal Changes in Cheese.—Many abnormal changes may occur in cheese that has been improperly made or produced from low-grade milk. Excessive production of lactic acid gives rise to a sour cheese; the excessive splitting of the fats to a rancid cheese. A malty flavor, due to the development of *S. lactis var. maltigenes,* is sometimes encountered. Bitterness may result from a certain type of protein decomposition. When the casein is greatly broken down the result is a putrid cheese. Such cheese may not only be repulsive but is dangerous because of the growth of putrefiers. "The fact that cheese is ripened by bacteria and that bacterial activity in the product is essential to the characteristic odor and flavor of the properly finished product does not remove the stigma of rot or decay from a product in which undesirable bacterial activity has produced odor, taste, or texture which is abnormal. Many products of this type are constantly observed and are properly designated as rotten or decayed and should be no more permitted on the market than rotten tomatoes or rotten fish." Such products may contain the *botulism* toxin in addition to many organisms which cause illness when eaten. Jordan found that nearly 4 per cent of the cases of food poisoning occurring in the United States during 1914–1915 were due to cheese.

Foreign organisms may find their way into the cheese and give rise to inferior products. Members of the *coli aerogenes* group produce gas. It is the gas that cause the many small holes in the cheese. At times the cheese may become so rounded in the center, due to the gas, that it rolls from the shelf.

Abnormal pigmentation which sometimes occurs in cheese is due to micro-organisms. This coloring of cheese may take the form of red, brown, black, yellow, green, or blue spots within or on the cheese.

The use of clean milk with a uniform microflora, if it can be obtained, would prevent abnormal changes in cheese; but where milk is obtained from many dairies greatly varying in the sanitation practiced, this is impossible. The ideal method would be to use pasteurized milk. With some varieties of cheese this

can be done successfully, but with others it cannot. The heat used in pasteurization may have an undesirable effect on the curd. Furthermore, our knowledge of the specific micro-organisms concerned in the ripening of the cheese is not complete enough in all cases to warrant the use of pure cultures. The starters which have been used with varying degrees of success are sour milk, buttermilk, whey, moldy bread, and the infusions of ripened cheese. Many of these are little better than the seeding of the original milk. For cheddar cheese pure cultures of various types of lactic acid streptococci, as well as of lactobacilli are used. The lactobacilli give a more highly flavored product than do the streptococci.

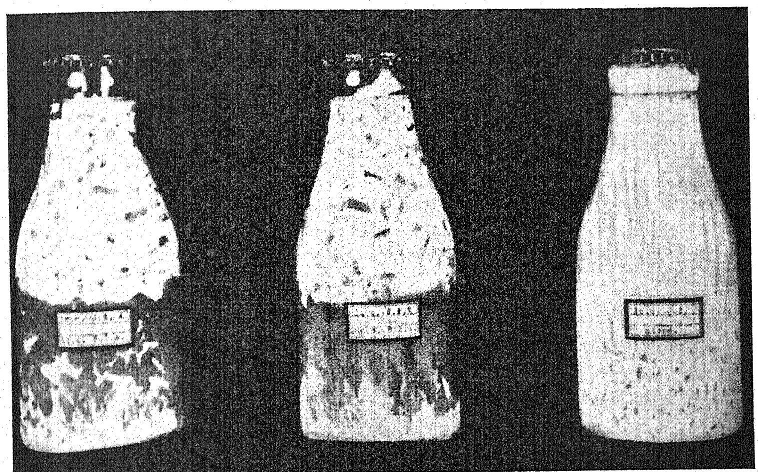


Fig. 93.—Illustrating the influence of gas producers in milk. (*After Hammer.*)

To overcome the variation which must of necessity occur from chance seeding, such as that mentioned above, and also to prevent infection and cheese poisoning, some advocate the pasteurization of the ripened product. This, when done at the proper temperature and under carefully controlled conditions, apparently gives a good product, uniform in texture and flavor, free from pathogens, and with good keeping properties.

ICE CREAM

The production of ice cream is a modern art. It has developed more rapidly in the United States than in other countries. In

this country alone over 350,000,000 pounds of milk are used annually in the production of ice creams. This is about twice the milk production in Japan. As defined by the United States Department of Agriculture: "Ice cream is a frozen product made from cream and sugar with or without a natural flavoring and containing not less than 14 per cent of milk fat. Fruit and nut ice cream must contain not less than 12 per cent of milk fat. When made from clean pasteurized products under sanitary conditions, it is a valuable and highly nutritious food. If made from unclean or infected milk, it becomes dangerous."

The enormous number of bacteria which may occur in ice cream may be seen from the work of Ayres and Johnson who in 1915 collected 91 winter samples and 94 summer samples of ice cream from retail stores in Washington. None of these samples contained less than 120,000 bacteria per cubic centimeter. The average for the 185 samples was 37,000,000, the maximum 510,000,000 and the minimum 120,000 per cubic centimeter. Later Hammer made a comprehensive study of ice cream, including an investigation of the source of bacteria, the possibilities of securing a low bacterial count, and the changes in the bacterial flora that take place during storage at low temperatures. He concluded that unpasteurized cream is the greatest source of bacteria, and may be responsible for the large bacterial count, and that sugar and vanilla extract are unimportant as a source of contamination. His investigation also showed the importance of the utensils as a source of bacteria. By sterilizing all utensils in an autoclave and by pasteurizing the cream at 60° C. for twenty minutes he was able to produce ice cream in 15 to 20 gallon lots with a bacterial content of 4200 per cubic centimeter. Bacteria can and do survive for long periods in ice cream. Even pathogens survive much longer in ice cream than in milk and other food products kept at room temperature. The longevity of *Eberthella typhosa* in ice cream is seen from the results reported by Purcha and Brannon in the table on page 319.

Epidemics of typhoid fever have actually been traced to infected ice cream. Cumming reported 23 cases which developed among twenty-nine persons who partook of ice cream at a school picnic at Helm, California, in 1916. Ice cream was the only food partaken of by all, and as the chocolate ice cream was the favorite flavor this was determined to be the source of the infection. This was because: (1) Those not partaking of it did not become ill, (2) those partaking of it but no other food became ill, (3) those eating chocolate ice cream were taken with

acute intestinal symptoms, and (4) those eating the largest quantity of chocolate ice cream were the most seriously ill.

Sample taken	Typhoid bacteria per c.c. ice cream
Before freezing	25,000,000
Freshly frozen	51,000,000
5 days old	10,000,000
12 days old	7,000,000
20 days old	2,000,000
70 days old	660,000
104 days old	900,000
134 days old	210,000
165 days old	640,000
170 days old	711,000
200 days old	60,000
260 days old	57,000
290 days old	53,000
342 days old	51,000
430 days old	30,000
544 days old	13,000
648 days old	11,000
2 year old	6,300
2 years, four months	Living typhoid germs present

Dysentery is also often spread by means of ice cream. Smillie studied 75 cases and found the etiology of them to be as follows:

	Cases
Contact with an acute case	21
Contact with a carrier	2
Contact with house cases	4
Condensed milk epidemic	15
Ice cream cones	9
Flies	6
Milk	1
Water	1
Fruit	1
Unknown	15

The dysentery bacillus of Flexner was actually isolated from the ice cream cones.

Hamilton has pointed out that ice cream epidemics can be

prevented by: (1) The use of ingredients with a clean sanitary history, (2) the use of properly cleaned utensils and a clean factory; and (3) the proper handling of materials by individuals with a clean bill of health. The first of these is to be controlled by the pasteurization of the milk and cream; the second, by frequent inspection of the plant; and the last requires regular and careful inspection of all workers for communicable diseases.

CHAPTER XXVIII

BACTERIA IN OTHER FOODS

ALL foods except those recently cooked or sterilized and then sealed contain micro-organisms. Most of these micro-organisms are saprophytes which cause food spoilage. Occasionally the food contains pathogens that may give rise to diseases in the consumer. The number and variety of germs contained in a food vary with its kind and history. Animal foods contain many micro-organisms, some of which may be pathogens, and may even multiply in such foods. Plant foods usually contain fewer micro-organisms, and are less likely to be disease producers. The pathogens seldom if ever multiply in vegetables and fruits; consequently, the danger from these foods is not as great as in the case of animal products.

Animal Tissues.—Veal, beef, pork, lamb, fish, and fowl are among the commonest animal products used as human food. The glandular tissues of the various animals are more complete foods than are the muscle tissues; yet, glandular tissues are more likely to convey disease than the muscle tissues, and should be inspected even more carefully and cooked more thoroughly than the muscle meats. However, this should not be taken to imply that raw or diseased muscle meat should ever be used as food.

The susceptibility of lower animals to diseases which are common to man varies with the species; hence, the likelihood of these animals conveying infection also varies. For example, goats, horses, dogs, and cats are quite resistant to tuberculosis and chickens are probably immune to anthrax; consequently, they do not convey them to man. Chopped meats are usually prepared from trimmings, leftovers, and inferior grades, consequently contain many bacteria. The extra handling adds to those already present and the hashing carries the bacteria through the entire mass which is an excellent medium for their multiplication. This is recognized by the fact that Weinzierl and Newton proposed a bacterial standard of not over 10,000,000 per gram for hamburger and found on a basis of this standard that 50 per cent of the samples examined had to be condemned. Hence, this form of meat is prone to produce illness.

Bacteria in Meats.—The tissues of healthy living animals are free from bacteria, but after death bacteria rapidly invade and at optimum temperature quickly decompose animal tissue. During the dressing and handling of meat bacteria are constantly being added. Some early western pioneers recognized this and killed and handled their beef and venison with great care. It was then hung in a cellar free from flies and dust and kept for weeks even during the summer months without spoiling.

Attempts have been made to set bacterial standards for the judging of meat. One authority suggested 1,000,000 bacteria per gram for the standard, and meat containing greater numbers he considered should be condemned. Others have tried to conform to this standard, but Carey showed that such a standard would condemn much of the ground market meat. At times he found that sausage often contains as many as 10,000,000 bacteria per gram. Most of the organisms occurring in meat are the common decay bacteria, but occasionally parasites and pathogens are present. The failure to find a direct relationship between bacterial counts and food spoilage and the indefiniteness of our knowledge concerning the specific causative organisms of meat poisoning have led to the rather generally accepted conclusion that bacteriological examination alone cannot be used as a criterion for the judging of meat. Antemortem and postmortem examinations of the animal and the general condition and history of the meat must all be considered in reaching a conclusion as to whether the meat should be used for human consumption or not.

Illness Due to the Use of Meat.—The excessive use of meat may injure the kidneys and possibly other organs of the body; furthermore, meat may carry bacteria or their products which may cause illness, or even death. The conditions under which meats may cause illness can be conveniently grouped under three heads:

1. Illness may be caused by eating meat from diseased animals.
2. Illness may be caused by eating putrid meats.
3. Illness may be caused by specific toxins produced in the meat, botulism.

It has been known for a long time that the flesh of animals dead from certain diseases or slaughtered while suffering from a disease is not a safe food for man. The ancient Egyptians understood this, as is indicated by the Mosaic Law: "Ye shall not eat of anything which dieth of itself." Yet emergency

slaughtering of ill or injured animals and the use of their tissue for food is not an uncommon procedure in some districts. Such meat is likely to cause illness in the consumer.

The more common bacterial infections which may be conveyed by meat to man are: Tuberculosis, anthrax, Malta fever, actinomycosis, paratyphoid, puerperal fever, pleuropneumonia, glanders, mucous diarrhea, and the illness caused by *Brucella abortus* so prevalent among cattle. The relationship between most of the above named animal diseases and human infection has been fully established, and the evidence is conclusive that in districts where uninspected meat is used they play an important rôle in causing human illness.

Gärtner in 1888 studied an outbreak of acute gastro-intestinal disease which occurred among a group of individuals who had eaten meat of a diseased cow. He isolated from the infected meat and also from the spleen of a fatal case *Salmonella enteritidis*. Since that day, this and related organisms have been repeatedly obtained in cases of food poisoning. The organisms all belong to the *Salmonella* group. It is now generally believed that they are the cause of much of the meat poisoning, and reach the meat by the following avenues:

1. The animal from which the meat was obtained may have actually been suffering with the infection at the time of slaughtering.
2. Both the *S. enteritidis* and *S. paratyphi* groups are often found in the excretions of healthy men and animals, and healthy carriers who handle the meats often infect them.
3. Uninfected meat may be piled with infected meat. In this way, the organisms become distributed through large quantities of meat. Moreover, these organisms under appropriate conditions of temperature multiply in meat, and a small initial infection may give rise to far-reaching effects.

It is often claimed that putrid meat causes poisoning, but the evidence for this is not conclusive. In the past many such cases were believed to have occurred and were attributed to ptomaines which were thought to be produced in the meat. Today it is doubtful if true ptomaines play an important part in meat poisoning. Certain putrefying bacteria, such as *Proteus vulgaris,* have repeatedly been obtained from putrid meat which has caused illness, and it is held by some that they are the cause of the illness. How they act is not definitely known at present. They may act, as suggested by Vaughan, by the production of "protein-split substances."

"The most characteristic examples of food poisoning, popularly speaking, are those in which the symptoms appear shortly after eating and in which gastro-intestinal disturbances predominate. In the typical group outbreaks of this sort all grades of severity are manifested, but as a rule recovery takes place. The great majority of such cases that have been investigated by modern bacteriological methods show the presence of bacilli belonging to the so-called "paratyphoid group" (*S. paratyphi*) or the Gärtner group (*S. enteritidis*). Especially is it true of meat-poisoning epidemics that paratyphoid bacilli are found in causal relation with them. Hübener enumerates forty-two meat-poisoning outbreaks in Germany in which bacilli of this group were shown to be implicated, and Savage gives a list of twenty-seven similar outbreaks in Great Britain. In the United States relatively few outbreaks of this character have been placed on record, but it cannot be assumed that this is due to their rarity, since no adequate investigation of food poisoning cases is generally carried out in our American communities."

Meat Inspection.—The quality and wholesomeness of meats and meat products produced and shipped to foreign countries and across state lines for domestic consumption are probably the best of the entire world. This is due to the administration by the Bureau of Animal Industry of the Federal Meat Inspection Act of 1906, supplemented by the Act of 1913. This provides for the adequate inspection of approximately 60,000,000 head of cattle, sheep, swine, and goats slaughtered annually in the United States. The inspection proceeds by logical steps, beginning with the careful antemortem examination of the animal, the inspection of the carcass while being dressed, the supervision of the curing, pickling, smoking, cooking, and canning of all meats, and finally with the proper, honest labeling of all meat products. It has been very aptly described as proceeding "from the hoof to the can," or "from the livestock pen to the finished meat or product in the labeled package ready for shipment to the consumer." The severe penalties provided in case of its violation put teeth into the act.

Approximately one half this number, 30,000,000 head, do not move in interstate or foreign commerce; hence they do not have the benefit of federal inspection, and must be looked after by state and city inspectors. Jones estimates that less than one third of our cities with populations of 5000 have any regulation of meat inspection, and that in many of these the inspection is incomplete. This is a distinct menace to the health in these

communities, for the producer soon learns that he can dispose of animals on the local market which cannot meet the rigid inspection for interstate and foreign commerce.

This condition can be remedied by having the cities and municipalities pass and enforce ordinances regulating these matters. These ordinances should, in so far as they are applicable, be modeled after the Federal Meat Inspection Law, and should provide for both postmortem and antemortem inspection of all animals to be used for human consumption and severe penalties for violation of the regulations. In small communities, where

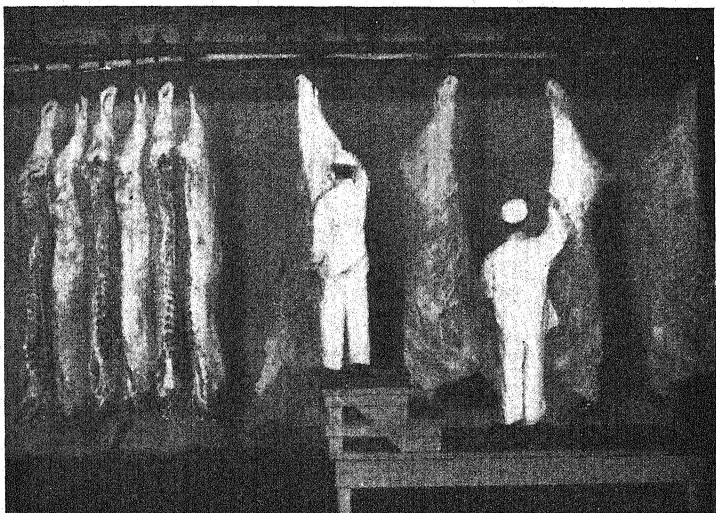


Fig. 94.—Final inspection of dressed beef. (*Courtesy, Swift and Company.*)

possible, expense can be reduced by cooperation in the passage and enforcement of the act. The city and county of San Francisco has an ordinance which may well be used as a model.

Prevention of Meat Poisoning.—Proper public meat inspection will do much to prevent meat poisoning, but the consumer must also cooperate. It is quite evident that spoiled meat should not be consumed, but it is not always easy to recognize putrefaction in the early stages. However, it is well to remember that the process commences at the surface and spreads to the deeper layers. There is a tendency for spoilage to first manifest itself along fibrous tissue or the bone. The cut surface of spoiling meats usually appears porous; indentation made with a blunt

object persists; the fat changes in color; the bone marrow becomes soft. There is a foul odor which is most marked about the bones and in the fat. The odor becomes more pronounced during cooking and does not disappear from the fully cooked meat.

Meat should be kept in a properly cooled refrigerator, and the refrigerator should be frequently and thoroughly cleansed with hot soda solution. Chicken, fish, and chopped meats are especially apt to spoil, and it is a good precaution to thoroughly cook all meat before it is eaten. This will destroy all pathogens and destroy or weaken most toxic bacterial products which may have been produced in the meat.

Fresh Vegetables.—The discovery of vitamins and the accumulated knowledge of their value in human nutrition have considerably increased the use of fresh vegetables in the diet. This is as it should be, yet it introduces an increased possibility of infection which should be recognized and guarded against. The edible parts of vegetables and leafy plants grown in or near the soil become covered with micro-organisms. If pathogens are in the soil, these also may adhere to the plants. In some foreign countries, where night soil is used as a fertilizer or where sewage is used for irrigation purposes, there is great danger of infection. In the United States the likelihood of pathogens being in garden soils is slight, except where human excreta is improperly disposed of and finds its way into the soil with the animal refuse used as fertilizers. There is also a possibility where sewage-laden waters are used for irrigating truck gardens.

Watercress grows rapidly in warm seep waters which may carry human pathogens. In rural districts, during the spring months, there is a tendency to gather this for human consumption, paying little or no attention to the nature of waters in which it is growing. Wary reports an epidemic of typhoid in a suburb, supposed to have been caused by eating watercress grown in beds fertilized with sewage. Another outbreak occurred in Philadelphia where 18 out of 19 persons who ate watercress sandwiches became ill with typhoid fever. Evidence pointed to the watercress as the cause. Similar outbreaks have occurred where other vegetables and leafy plants apparently were the cause.

There is an opportunity for vegetables to gather more germs, occasionally pathogens, from the hands of the venders. If the outer leaves of lettuce and cabbage are left intact until the product is prepared for serving they keep better and the inner

parts are protected against contamination. Tests have shown the outer leaves to carry many more micro-organisms than the inner leaves.

Bacteria do not penetrate the living tissue and soon die when mechanically introduced. However, they do adhere tenaciously to the surface of the plant and are not completely removed by washing. This is sometimes vividly demonstrated in the laboratory by infecting some of the leafy plants with a pigment-producing micro-organism (for example, *Serratia marcescens*), after which the leaves are carefully washed and plated on a suitable medium. After a time red spots appear on the plates which are due to the growth of the added contaminant. Inasmuch as ordinary washing is not sufficient to free vegetables of germs, chlorination has been suggested. Tests conducted in Japan where night soil is used showed that *Eberthella typhosa* on vegetables were not killed in forty minutes in solutions having 200 p. p. m. of chlorine. Vegetables such as greens, radishes, turnips, and spinach, were not disinfected with bleaching powder, irrespective of its concentration or length of exposure. Failure to disinfect is probably due to lack of penetration.

Fresh Fruits.—Fresh uncooked fruits carry a varied microflora, depending upon a number of factors. Small soft fruits and overripe fruits attract flies and insects which convey to them germs, some of which may occasionally be pathogens. Fruits rich in acids are more prone to mold spoilage, whereas those rich in carbohydrates are attacked more readily by bacteria. Fruits, such as the berries, growing near the earth become covered with micro-organisms. These may be added to by the unclean hands of careless pickers. Fruits exposed for sale at streetstands, unless properly covered, may harbor flies. Abbott found pathogenic bacteria and other micro-organisms of sanitary significance on grapes and berries exposed for sale at sidewalk stands. Juices from acid fruits have a slight bactericidal action against *E. typhosa,* and the skin of many fruits if not overripe protects the inner part against pollution. Consequently, washing and peeling materially reduce any danger there may be from the use of fresh fruit.

Confections.—"The confections are members of a large group of foods which are often handled in such a manner as to cast doubt on their healthfulness. They are often dispensed by dirty hands, from receptacles, and kept in stores of questionable cleanliness. They are usually eaten without further preparation which would render them free from undesirable bacteria,

should they be present." The poorest grades and often the least sanitary forms are consumed by children who are highly susceptible to both the poisonous ingredients and any infection which they may carry.

Tanner and Davis analyzed thirty samples of candy which they purchased on the open market. The bacterial content ranged from 0 to 2,240,000 per cubic centimeter. They found that the bacterial content depended more upon the presence of certain constituents than on the method in which they were dispensed. Fudge, and those containing soft centers, chopped figs, and cocoanut had high bacterial contents. The real hard candies were lower, and candies containing peppermint and other essential oils were still lower, due to the germicidal properties of the flavor. Even frail micro-organisms do not readily die in or on candy. Fudge was prepared in the ordinary manner and then heavily seeded with cultures of *Escherichia coli* and *Serratia marcescens*. After four months' storage under dry conditions living cells of both organisms were isolated. Cummings found that *Eberthella typhosa* when inoculated in large numbers into chocolate survived for one hundred and forty days. Such experiments indicate that candy should be handled under sanitary conditions and by healthy individuals; otherwise, it is a menace to the public health, for disease carriers, if allowed to prepare or vend candy, may readily spread typhoid, tuberculosis, or other diseases.

Explosions in Chocolate Candies.—The chocolate coating on candies sometimes cracks and at times is broken into a number of fragments. This occurs in from ten to fourteen days after the candy has been manufactured, and apparently the manufacturer is unable to foretell the trouble. It occurs only in chocolates having a fondant center, which is composed of egg white, sugar, flour, flavoring, and other constituents. It has been shown that this spoilage is due to the growth of gas-producing micro-organisms within the candy. These bacteria have their origin in the egg, starch, and other constituents used in the making of the candies. The organisms which have been incriminated in this connection are the various gas producers: *Escherichia coli, Clostridium sporogenes,* and *Cl. multifermentans.*

CHAPTER XXIX

BOTULISM

The most fatal form of food poisoning known comes from the ingestion of toxins elaborated in the food by *Clostridium botulinum*. It was early associated with sausage poisoning from which it derived its name. However the name has lost its original significance, for it has been shown that the organism grows and produces its toxins in many other foods. It is unique in being a very resistant spore-bearing anaerobic saprophytic micro-organism producing one of the most potent toxins known.

Historical.—For many years botulism, or as it was first known, sausage poisoning, has been distinguished from other forms of food poisoning. This has been due to its high mortality, its peculiar symptoms, and its association with the consumption of sausage.

For nearly two hundred years Würtemberg, Germany, was the reputed birth-place and home of sausage poisoning. Varied were the interpretations which were given as to the cause: Some thought it to be due to copper which was supposed to have come from the vessel in which the sausage was prepared; while others believed it to be caused by belladonna or henbane which had been added accidentally or intentionally. In 1892 official instructions were issued concerning the preparation of sausages. In these it was held that metallic poisons play no part in the poisoning, but the poison was carried into food by various imported condiments used to give it flavor. In the twenties of the nineteenth century, a German poet and medical writer, Kerner, enumerated 174 cases with 71 deaths which had occurred in various epidemics in Würtemberg. He considered they were due to the great consumption of large sausages which had been made by primitive methods from inferior materials; consequently, it came to be suspected that some deleterious change occurred within the sausage itself, thus rendering it poisonous. One writer attributed it to a poisonous fatty acid, another to acrolein, and still another considered that there was produced within the sausage hydrocyanic acid and that this was the cause. It was even suggested by some that the meat had come from sick ani-

mals, and the cause of the trouble lay there. There were also those who suggested that toxic substances were produced in the meat during the smoking. However, the true etiology of botulism was not established until 1894 when Van Ermengem, following an epidemic in Belgium, obtained from the suspected food and from the spleen of one of the victims, *Cl. botulinum.* Since that time it has been repeatedly demonstrated that this is the causative organism.

Botulism was long regarded as a rarity in America, but since 1912 numerous cases have been recognized, as may be seen from the following table by Geiger, which gives a record of human botulism in the United States between 1920 and 1925.

Year	No. of outbreaks	No. of cases	Deaths	Mortality (per cent)
1920	14	48	33	68.7
1921	14	56	22	39.3
1922	21	55	46	83.6
1923	12	21	16	76.2
1924	8	43	29	67.4
1925	8	21	15	71.4

The outstanding characteristics of these cases are the high mortality, the rather restricted areas in which they have occurred, and the fact that most of them have been caused by home-processed foods. Probably the cases were greatly increased by the extensive use of the cold-pack method which came into favor during the war. It is just possible that many of the earlier so-called cases of "ptomaine poisoning" reported in this country were botulism.

The occurrence of recognized cases in the United States has stimulated research in this field, which has shown: (1) That botulism is endemic within regions of the United States. (2) It results from the ingestion of certain improperly processed fruits and vegetables in addition to meats. (3) Forage poisoning in horses and other animals may at times be due to botulism. (4) The causative organism is a saprophyte and may occur in native soils. (5) There are different strains of the organism which, although similar morphologically, produce specific toxins.

Causative Organism.—*Clostridium botulinum* is a large, spore-bearing anaerobe. It is usually described as being from 4 to 9 microns in length and from 0.9 to 1.2 microns broad. Generally

the ends are rounded, and often two or more individuals are attached end to end, occasionally forming long threads. It is

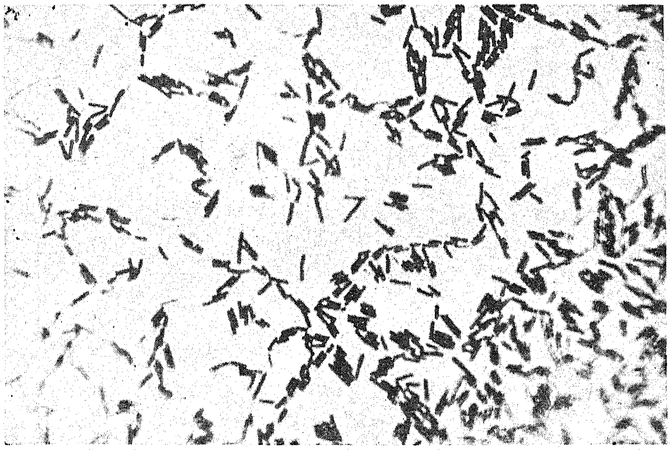


Fig. 95.—Clostridium botulinum. (*Courtesy, Dr. Dozier.*)

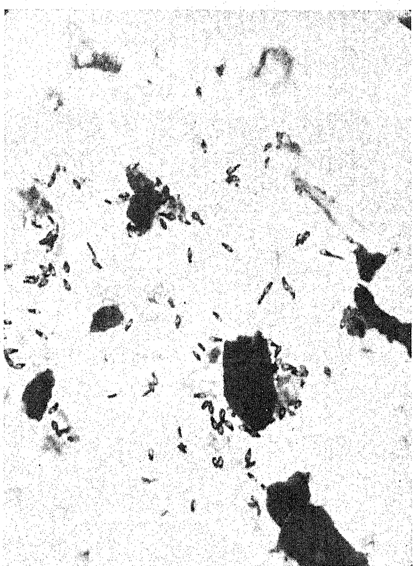


Fig. 96.—Clostridium botulinum showing spores. (*Courtesy, Dr. Dozier.*)

motile with 4 to 8 flagella at the pole, and forms resistant subterminal oval spores. Three types are known: Type A, type B,

and type C which differ in resistance, toxicity, agglutinative and proteolytic powers but are the same morphologically. The maximum heat resistance of numerous strains tested by Meyer in a neutral phosphate solution was found to be:

4 minutes at 120° C.
10 minutes at 115° C.
100 minutes at 110° C.
330 minutes at 100° C.

The thermal death point of type C is considerably below that of the other types. Their resistance to heat varies with the specific medium in which they are heated and its hydrogen ion concentration. Spores of *Cl. botulinum* exhibit approximately the same resistance when heated in ripe olives with a *p*H 7.93, corn *p*H 6.35 and spinach *p*H 5.05 whereas in asparagus with a *p*H 5.25 to 5.55 they are more readily destroyed. In fruit juices with a *p*H below 4.5 the hydrogen ion has a decided influence on the destruction of *Cl. botulinum.*

Although *Cl. botulinum* is an anaerobe and grows best when all oxygen is excluded, yet it grows well under aerobic conditions in the presence of other organism which consume the oxygen. Thus, it may grow and elaborate its toxin in other than canned foods. The organism grows well in a great variety of media, and meat extracts are not essential for its growth and toxin production. It grows readily at temperatures ranging between 20° and 40° C., but the greatest toxin production takes place at approximately 37° C. Small quantities of sodium chloride seem to be essential for its growth, but large quantities are injurious as growth ceases in a 6 per cent salt solution, but not in 50 per cent syrup.

Distribution.—*Clostridium botulinum* is widely distributed in nature. Burke found the organisms in the following substances: (1) Bird-specked, bruised, and moldy cherries; (2) bean leaves covered with spots or droppings of insects or small animals; (3) spiders from bush bean plants as well as on the bush bean; (4) moldy hay upon which horses and mules had fed and later developed botulism. Although Burke's work was confined to California, other workers have shown that the organisms are widely distributed elsewhere. It is thus possible to contract botulism from the eating of a great variety of improperly processed foods from many localities.

Meyer and his co-workers made the surprising discovery that the organisms occur most frequently and most abundantly in

virgin soil. They were found in soil from mountain sides where there was no evidence of soil pollution by either man or animal. They further found that type A is more prevalent in virgin soil, whereas type B predominates in soil that has been cultivated. The districts most heavily infected by type A as shown by the work of these investigators are the mountainous and coast states of the West, including Washingon, Oregon, and California, with Utah, Wyoming, and Montana slightly less infected. Type B seems to predominate in the East. These findings led Meyer to conclude that the original and natural habitat of *Cl. botulinum* is mountainous virgin soil, from where the organisms are transported to the lowlands by water, wind, and birds. As a result of the changed environment, there is a mutation of type A to type B. The organisms have been found on fruit and vegetables in the infected districts to the extent of 30 per cent of the samples tested. It is the toxin which causes illness; hence, there is no danger in eating fresh fruits and vegetables even when they carry spores of *Cl. botulinum,* since they are not accompanied by the toxin.

It was believed that the organisms grow and produce their toxin only in meat, until Dickson showed that fruits and vegetables are also suitable pabulum. Since then a number of outbreaks have occurred, resulting from the consumption of improperly processed beans and olives. The organisms apparently grow well and elaborate a strong toxin in silos, for it has occasionally been the cause of forage poisoning.

Recently, after an extensive study of "western duck sickness," Kalmbach and Gunderson conclude, from the following reasons, that the disease is due to a toxin produced by *Cl. botulinum* type C: (1) The clinical picture of the disease in the field and that caused by administering toxin produced by pure cultures of type C *Cl. botulinum* are similar. (2) The causative organism was frequently obtained from the tissues of affected birds but was absent from the tissues of healthy birds. (3) The toxin was obtained from food and water commonly ingested by birds. (4) The incidence, course, and disappearance of duck sickness in the field is in keeping with the theory that the disease is due to *Cl. botulinum* type C.

This organism finds ideal conditions for growth and the elaboration of its toxin in the organic matter covering the bottoms of shallow, warm, alkaline waters, which occur in the regions where this disease is prevalent. No satisfactory solution for the disease has been offered and the toll of death varies widely from

year to year. It is highly probable that it is one of the major causes of death among waterfowls, and it is conservatively estimated that the loss of ducks at the north end of Great Salt

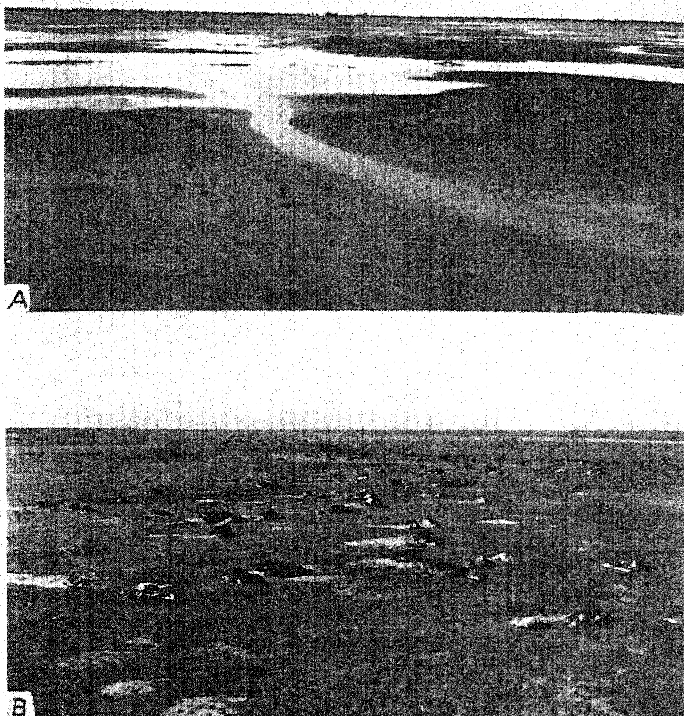


Fig. 97.—*A,* Typical duck-sickness environment; shallow, stagnant water and alkaline mud flats; *B,* severe outbreak of the disease on the south side of Willard Spur, Utah, in 1932. On November 12, for 6 to 8 miles, there were 8000 to 10,000 dead ducks to the mile of shore line. Many additional thousands lay scattered along the shores of Bear River Bay. (*After Kalmbach and Gunderson, Tech. Bull. 411, Dept. of Agr.*)

Lake during the summer and fall of 1932 was a quarter of a million. One redeeming feature is that the toxin apparently may be ingested by man without ill results.

Pathogenicity.—It is generally stated that *Cl. botulinum* does

not multiply and elaborate its toxin in the bodies of animals, and clinical observations confirm this view. However, recent laboratory experiments have showed it possible to produce fatal botulism by feeding massive doses of toxin-free spores, thus raising anew the question: May spores germinate within the body and form a virulent toxin? If they do, there is a possible danger in the eating of food which contains many spores of *Cl. botulinum* even though the toxin has been destroyed by cooking.

Botulism, due to the ingested poison, may occur not only in human beings, but also in horses, mules, and cattle. Chickens often die in large numbers after feeding upon discarded food containing the toxin. The peculiar symptoms which develop have caused the malady to be referred to as "limberneck."

Botulism in cats, dogs, hogs, and goats is rare, though cases have been reported. Rabbits, guinea-pigs, mice, and apes are susceptible to experimental injections or feeding of the toxin.

Symptoms.—The symptoms develop only after an incubation period of from twelve to twenty-four hours, the time varying with the quantity of toxin ingested. The most constant and characteristic symptoms are: (1) The mouth and throat become dry; the skin is usually dry at first, but later is rough and harsh. (2) The motile activity of every part of the alimentary tract is lessened; there is usually constipation and retention of urine without vomiting. (3) There is a general enfeeblement of all voluntary muscles. (4) There is an absence of fever and little interference in the early stages with the higher nervous centers; hence, no delirium. (5) In many instances there are scintillations before the eyes, dimness of sight, and double vision. Often this is the first indication the patient has that anything is wrong. (6) There is a dropping of the eyelids, and there may be respiratory or circulatory disturbances which threaten or actually end life. Loss of voice and difficulty in swallowing is often manifest. (7) The pulse is usually slow, but recent cases in the United States report a rapid pulse, in some instances as high as 150 per minute.

The duration of the disease varies widely. Cases have been reported which have lasted as long as one hundred days. In fatal cases death usually occurs about the tenth day. The mortality of botulism is high. In the United States, as stated by Dickson, it has been over 64 per cent.

Toxin.—The toxin is produced under strictly anaerobic conditions. However, when grown in mixed cultures, the organism may produce its toxin because the associated organisms

withdraw the oxygen from the vicinity of the anaerobe. Although it will grow and produce toxin in a slightly acid medium yet, according to Dickson, it is much more potent if grown in an alkaline medium and in the dark. In the early history of the subject, it was believed that animal protein was necessary for the production of toxin. Later work has shown that this is not necessary as the toxin is readily produced in string beans, peas, other vegetables, and even some fruits. The best temperature for its production is between 28° and 37.5° C. It is destroyed when exposed to a temperature of 80° C. and also by sunlight and air, but will maintain its potency for six months if kept in the dark away from oxygen as would be the case in preserved food.

Botulinus toxin is one of the most potent poisons known. Van Ermengem reports an outbreak where 200 Gm. of poisonous beans caused the death of a human being. He cites another case in which a piece of preserved duck the size of a walnut caused the disease to last six weeks. In other cases given by him a patient died after tasting a small spoonful of spoiled corn; another died after "nibbling" a portion of a pod of spoiled string beans. Still another was made severely ill by simply tasting but not swallowing a pod of spoiled beans, all of which indicates that it is not safe to even taste meat or vegetables which are suspected of being spoiled.

The toxin has never been obtained pure, but investigators have prepared it so potent that 0.000,001 of a cubic centimeter would kill a 250-Gm. guinea-pig in four days. It is so effective that only a few molecules are required to kill small experimental animals. Type A produces the most potent toxin. One author gives the ratio of lethal dose for type A, B, and C as 1: 50: 125. It is the only bacterial toxin known that is not destroyed by the gastro-intestinal secretions. The toxin may be absorbed into the body from the mouth or the intestines, but probably the greater portion enters from the stomach.

Antitoxin.—The toxin of *Cl. botulinum* is similar to that produced by the tetanus and diphtheria bacilli in that it is a true exotoxin, and if injected into animals in small increasing doses produces immunity. This has been made use of in preparing an antitoxin which is quite effective in protecting experimental animals against botulism. Where it has been used on human individuals the results have been encouraging, but the number of cases in which it has been used is small. The antitoxin is effective only when administered previous to the onset of symp-

toms; hence, it does not neutralize the poison after it has once combined with nervous tissue. It appears to have some effect when taken through the stomach but is most effective when given directly into the blood stream. The antitoxin produced by one type protects only against itself; consequently, the type must be determined, or the antitoxin for all three types given.

Prevention.—The prevention of botulism at the present time rests upon the following principles: (1) Only sound unspoiled fruits and vegetables should be used for canning. These should be handled in a sanitary manner and processed with reliable methods. The cold-pack method is not reliable. Complete sterilization results only with the pressure method, and then the time and temperature must be such that the entire content of the can is sterilized. The latter naturally vary with the size of the container and the nature of the products. Where products are preserved by means of salt, not less than 10 per cent of salt should be used. (2) The housewife should learn the signs of spoilage and carefully examine every jar before using its contents. The signs of spoilage are: (*a*) The blown lid, the spurting of liquid when the cap is removed and the presence of gas bubbles in the jar. (*b*) The odor of butyric acid (rancid cheese), becoming more pronounced on standing. (*c*) The mushy appearance of the solid parts of the food in the jar. All cans presenting any or all of the evidence mentioned above should be destroyed. It is not safe to taste the product to find out if it is spoiled. (3) All food processed by unreliable methods and those suspected of spoilage but showing no sign should be boiled for thirty minutes before using or even before tasting. (4) Salads should not be prepared and then kept for long periods at room temperature before using. The toxin is not produced at low temperatures. The careful systematic application of these principles will greatly reduce if not entirely eliminate botulism.

CHAPTER XXX

FOOD PRESERVATION

MODERN thickly populated cities have been made possible through the development of rapid means of transportation and the extensive preservation by physical and chemical means of perishable foods. Today food is quickly transported hundreds or even thousands of miles from the producer to the consumer. The bounteous harvests of one season are preserved for the season of scarcity. The tables of civilized man today bear a greater variety of food than ever before. Modern developments have made possible the serving of Australian meats in London, California fruits in New York, Wisconsin milk in Manila, and eggs from China in Chicago. Today man receives a greater variety in his diet than at any previous period, and were it not for the ever-growing tendency to use highly refined food products this would guarantee to him a more nearly balanced diet. However, the greater distance between producer and consumer, which increases the opportunities for infection, and the more general use of food preservatives multiply the sanitary problems which confront the health workers.

Methods of Preserving Food.—Food may be preserved by heat, cold, drying, concentration, and chemicals. The various methods and foods usually preserved by each method are given in tabular form below:

I. Preservation by Temperature Changes:
- 1. Heat:
  - (*a*) Boiling (most fruits and some vegetables in glass containers).
  - (*b*) Steam pressure (vegetables, milk, etc., in tin cans).
  - (*c*) Pasteurization (market milk, beer, wine, fruit juices).
- 2. Cold:
  - (*a*) Freezing (meats, milk, ice cream, etc.).
  - (*b*) Refrigeration (low temperature but not frozen) (vegetables, fruits, beverages, meats, dairy products, etc.).

II. Drying:
- 1. In natural state (raisins, currants, and other fruits).
- 2. In powdered form (eggs, gelatin, milk, etc.).
- 3. After curing (various meats).

III. Concentration:
  1. Sugars (jellies, syrups, jams, preserves, milks).
  2. Salt (fish, meat, vegetables).
  3. Pickling (pickles, sauerkraut, beans, etc.).

IV. Chemicals:
  1. Spices and essential oils (fruit cake, mince meat, condiments, etc.).
  2. Other organic chemicals referred to as food preservatives (many foods may be preserved by the use of benzoate of soda, sulphurous acid, etc.).

V. Excluding of oxygen:
  1. Eggs, fruits, etc.

VI. Filtration:
  1. Liquids, including media of various kinds.

Heat.—The preservation of food by heat is nearly as old as the use of fire for the cooking of food. However, in the early days it was used only occasionally and then in a haphazard way. It was given a great impetus by the work of Pasteur. During the middle of the nineteenth century the French wine industry, which had an annual value of five hundred million francs, was threatened by what was known as the "disease of wine." The wine industry faced a crisis, and it appeared that French wines were about to lose their world-wide reputation for flavor and bouquet. Pasteur moved his laboratory into the heart of the afflicted district and after much experimentation found that heating the wine to a temperature of 55° to 60° C. for a few minutes prevented it from spoiling without harming the delicate flavor of the product. This method was tested on a considerable scale by order of the Naval authorities. The ship, Jean Bart, before starting on a voyage took on board 200 liters of wine, half of which had been heated under Pasteur's direction. At the end of ten months the heated wine was mellow and had a good color, whereas the other had an astringent almost bitter taste. Other more extensive tests with wine gave the same results. In this manner began the modern method of pasteurization which saved for France not only much money but has since also saved untold thousands of human lives throughout the world as a result of the commercial pasteurization of milk.

Pasteurization is carried out in the modern milk plant by heating the milk to a temperature of 65° C. and holding it at that point for thirty minutes. Such a procedure guarantees to the consumer a milk free from pathogens. In modified forms it is also used in treating cream, wine, beer, fruit juices, and vinegar.

Heating to the boiling point is a method quite generally used in the home for the preservation of fruits and to a lesser extent of vegetables. This may take the form of the cold-pack or open-kettle method, the efficiency depending to a great extent upon the nature of the product and the skill of the operator. Acid-containing foods are more effectively handled by these methods than are the neutral products. Usually the finished product is not sterile, but the anaerobic conditions under which

Fig. 98.—Aluminum pressure cooker, very acceptable for home canning.

it is kept prevents the germination of all molds and many of the bacteria.

Often the intermittent method of sterilization is used to increase effectiveness. This consists of heating the containers with their contents on three successive days, thereby permitting the germination of spores which escaped the first or even second heating. But even this does not insure a sterile product as the time and conditions are not favorable for all the micro-organisms

canning is usually done by means of steam under pressure. Food poisoning which has occurred during recent years has made some workers advocate this method even for home canning.

The temperature necessary for an effective "process" varies with a number of factors, chief of which are: (1) The nature of the food. Acid products are more easily processed than are neutral products. (2) The size of the can and the nature of the fill. (3) The rate of heat penetration for the particular product.

Even commercially canned foods are not always sterile, for it has been repeatedly shown that even cans which show no signs of spoilage contain living micro-organisms. Most of these are in the spore state and are restrained from growth by the anaerobic conditions. The aim of the manufacturer is to place on the market a product which will keep and also appear as attractive as possible. The growing demands for an attractive product has led in some cases to the use of glass containers. This may be accompanied by some danger, as recent work has shown that many of the cheap glass containers on the market will not stand the high temperature of processing without chipping. Bits of glass may thus find their way into the food products. The principles underlying successful home canning and the use of canned products is summarized by Thom as follows:

1. Only clean, fresh, sound products should be used for canning. It is hard to sterilize dirty or partly spoiled products such as stale, fermenting, souring, heating, slimy, wormy, specked, or partly rotted fruit or vegetables.

2. Keep a record of every can, its contents, and methods of handling.

3. Hold all cans under observation in the kitchen or pantry until you are sure that they are keeping. This means for a period of not less than ten days.

4. Some cans will spoil on account of faulty containers, accidents, under-cooking, or mistakes. Before even tasting, inspect every can; destroy the bad, recook cans of that lot or cans treated in the same way and apparently good.

5. Inspect at opening time:

In Tin

1. Both ends should be flat or curved slightly inward. Neither end should bulge, snap back when pressed, nor feel loose. *Make no exceptions.*

2. All seams should be tight and clean, with no trace of leaks.

In Glass

1. The cover should be firm—flat or concave with seam, rubber rings, and label clean and free from all signs of leaks.

2. The contents should appear free from molds, disintegration, cloudiness, or other abnormality, and show no discoloration when opened. Suction inward is highly desirable. No outrush of gas or spurt of liquid should occur.

The odor, observed immediately, should be characteristic of the product. No trace of a foreign or objectionable odor should be present. No disintegration, no mold, or other abnormal appearance should be observed. Liquid enough to cover the food is desirable in most products.

The inside of a tin can should be clean and bright or well lacquered, not extensively blackened or markedly corroded. If you know it is spoiled, destroy it. If after examining it, you cannot tell, add half the volume of boiling water and boil thoroughly. Canned vegetables should be boiled as an added precaution.

Cold Storage.—An ideal method for preserving food should retard natural metabolism in the food product to such an extent that it does not become over-ripe during the storage period. Furthermore, it should prevent bacterial changes without impairing the appearance, flavor, or nutritive value of the product. These conditions are more nearly met by refrigeration than by other means of food preservation. Cold, as a rule, does not destroy bacteria, for they will survive very low temperatures, but foods kept at low temperatures for considerable periods may show a decrease in the harmful bacteria. Although refrigeration kills animal parasites, it is often not sufficient even to prevent multiplication of micro-organisms, for Brooks in studying meat in cold storage isolated several organisms capable of growing at temperatures of —6° C. Other organisms have been found to grow at temperatures even lower than this provided the medium in which they are held does not crystallize. Low temperature has a selective action and prevents the growth of pathogens. Rosenau found that the organisms growing in milk at low temperatures are mainly putrefiers, and doubts that they produce toxic products. Refrigeration retards, but does not completely stop bacterial changes. The slowing down of bacterial multiplication is seen from the following results given by Russell. The time given is that required for a generation of *Escherichia coli*.

45° C. 20 minutes	25° C. 40 minutes
40° C. 17 minutes	20° C. 90 minutes
35° C. 22 minutes	15° C. 120 minutes
30° C. 29 minutes	10° C. 14 hours 20 minutes

From this we see that cold not only reduces the number of micro-organisms produced, but in addition it very materially retards the speed with which they act upon the product. The psychrophilic organisms are able to function at much lower temperatures than *Escherichia coli,* and it is quite probable that it is these micro-organisms which bring about the major changes occurring in cold storage foods.

It is evident that the effectiveness of food preservation by this method depends upon the temperature maintained. The home refrigerator, which is often placed for convenience in the kitchen near the range, may have a temperature near optimum for incubation of some micro-organisms. Where ice is used for refrigeration, the temperature seldom reaches zero. However, with the modern mechanical means much lower temperatures are maintained. The temperature to be used depends to a great extent on the products to be kept. Some foods such as ice cream and fish must be kept at temperatures below zero to maintain the texture and to prevent spoilage. Eggs, fruit, and similar products must be kept at a higher temperature, for low temperatures cause deterioration of the products.

The temperature at which fruit and vegetables are stored varies with the product, but as a general rule it may be stated that the best temperature at which to hold the storage room is just above the temperature at which the fruit freezes. Some vegetables, for example squash, may keep better at a higher temperature. In all cases ventilation and humidity must be carefully controlled if the best results are to be obtained. Fruit is often wrapped to prevent wilting and the spread of fungi.

Ice cream is usually stored in the hardening room which has a temperature of about —9° C., whereas meat may be stored slightly above or just below freezing temperature. The higher temperature permits the ripening processes to go on and does not interfere with the normal texture and appearance of the meat. Meat treated in this manner should be stored in a dry atmosphere so that moisture will not collect on its surfaces and permit the multiplication of bacteria. When frozen, the texture of meat, as well as its appearance and flavor are changed. Frozen meat on thawing rapidly spoils due to the oozing out of the serum, the looser texture, and to the rapid multiplication of the organisms which have slowly penetrated and seeded the frozen meat.

Fish is successfully stored for considerable periods only when frozen. Still better results are obtained by dipping it into water

and refreezing so that the surface is covered with a layer of ice. This prevents the growth of psychrophilic bacteria at the surface.

According to Pennington from 75 to 90 per cent of all the poultry produced in the United States is placed in cold storage, most of it for only a few weeks or months, but occasionally it is stored for years. The United States Department of Chemistry has shown that under such long storage the tissues become thickly seeded with bacteria and undesirable changes occur. These are described by Pennington as follows:

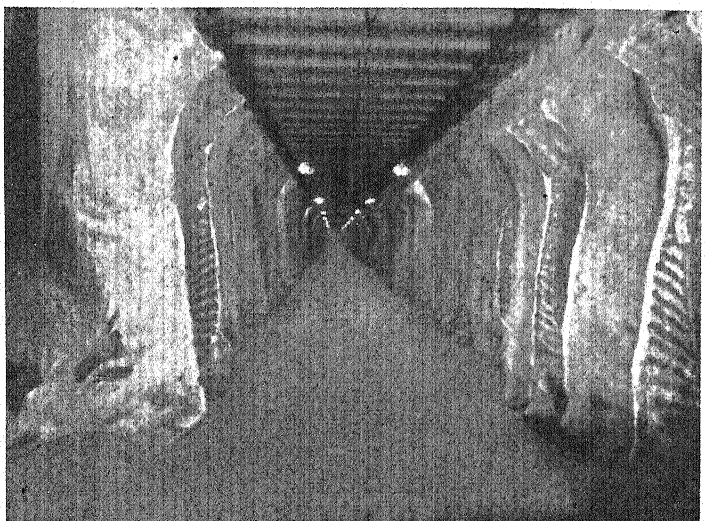


Fig. 99.—Beef hung in chilling room. (*Courtesy, Swift and Company.*)

"In the cold storage of chickens it is generally conceded that the fowl should be frozen as soon as possible. Some warehouses place them for a few hours at a temperature of $-23°$ C. and then transfer to a temperature of $-9.5°$ C. for permanent storage. Special care is used in the thawing when brought out of cold storage.

"The most striking difference between this chicken stored for three years and those stored for shorter periods, or those which are fresh is a pronounced inflexibility and the general green tint of the skin. The whole appearance of the bird was unpleasant in the extreme. The odor was not that of putrefaction, but was

of a sharp, penetrating, unpleasant character, having a biting property, which suggested the effect of acrolein on the eyes and nostrils." The skin, muscles, and internal organs all showed profound decomposition changes.

Drying.—Preservation of food by drying was one of the earliest methods used, and within recent years has been greatly extended and improved. This is exemplified in terms used to refer to the various preserved food products. The term "dried fruit" is applied to any product in which the moisture is reduced by exposure of the fresh material to the heat of the sun, whereas when the moisture is removed by artificial means the product is known as an evaporated or less often as a dehydrated or desiccated fruit. The term "dehydrate" is used to imply the removal of water without materially altering the constituents within the food product. By this means many fruits, vegetables, meats, and even the highly perishable products, milk and eggs, may be preserved.

Drying when compared with refrigeration has many distinct advantages and only a few disadvantages. The advantages are: (1) It yields, as Banks points out, a convenient product. "The possibilities in drying food products are enormous. One case may be cited. In preparing a vegetable soup or stew, the cook may choose from a dozen vegetables, picking out whatever combination or quantity is needed for the particular dish wanted. Powdered onion, garlic, mint, peppers, and other flavors are quickly available by the same method. Any quantity may be used whenever it is wanted." (2) The removal of great quantities of water greatly facilitates storage and shipment. For this reason dehydration of foods was greatly accelerated by the World War. It is especially suitable for products such as milk which may have to be shipped great distances. (3) All bacteria require moisture for multiplication, and if desiccated soon die. The time they can survive varies with the medium, extent of desiccation, and the specific micro-organism. Most pathogens are quite susceptible to drying. (4) Food products sufficiently dried and sealed may be kept for indefinite periods under great climatic variations without deterioration. (5) Drying is without effect on the nutritive value of most foods. Vitamins A and B are not affected, but the oxidation process going on in the dried product may reduce the efficiency of vitamin C. The disadvantages are: (1) The removal of the water may at times render certain constituents insoluble. Hence, it is not restored to its

original condition by the addition of water. However, attempts are being made to overcome this in the modern means of dehydration. (2) Some products on dehydration lose in appearance and flavor, many fruits become dark. These are often bleached by sulphur fumes. The sulphur also acts as a disinfectant and assists in the prevention of the growth of micro-organisms. It may, however, be objectionable in that it renders the food less digestible.

Fruits.—The ease with which fruits are preserved by drying varies widely with the variety, depending upon the concentration of the soluble constituents held within their juices. Grapes and prunes keep well when containing considerable water. Apples, peaches, and apricots keep less easily, whereas some berries and other fruits are dried with difficulty. Raisins and currants are best dried by the heat of the sun, whereas some products can be successfully dehydrated only by artificial means. The great objections to natural drying are dust, insects, and the uncertainty of the weather. With modern machinery it is possible to peel, core, and slice apples at one operation. They are then dipped for a few minutes in a weak solution of salt water to prevent the discoloration which would otherwise occur when the flesh of the apple is exposed to the air. According to Langworth, "After dipping, the apples are commonly placed in the drying trays, in which they are later taken to the drying machine. Many manufacturers subject them at this stage to a short fumigation with sulphur the purpose of which is to make the color lighter and to kill any moth eggs or injurious micro-organisms which may be present in the fruit. Sulphuring, which is used with various kinds of fruits, is in this country carefully regulated so as not to harm or harden the fruit, which when dried should be soft and pliable; on being removed from the desiccator the fruit is allowed to stand, for what is known as the 'sweating,' to take place, a process which usually requires several days and is carried out in the open air or well-ventilated chambers." Only fruit free from spoilage should be used for drying and the finished product should be handled in a sanitary manner.

Meats.—Meats may be dried either directly or after some preliminary treatment. In the hot districts of South America and the western plains of the United States, beef is often cut into strips and then exposed to the heat of the sun until sufficient water is removed so that it will keep. At other times it is

dehydrated by artificial means and placed on the market as a powder. Either product keeps well and is highly nutritious.

Pickling.—Cabbage is sliced into large vats and covered with layers of salt in the preparation of sauerkraut. The salt serves a two-fold purpose: (1) Due to the osmotic pressure it extracts the sap from the cell. This extract offers a very suitable medium for the growth of the lactic acid bacteria. (2) The growth of competitive micro-organisms are checked, whereas the lactic acid organisms are not influenced by the concentration of salt used (about 2 per cent). Hence, there results a microflora com-

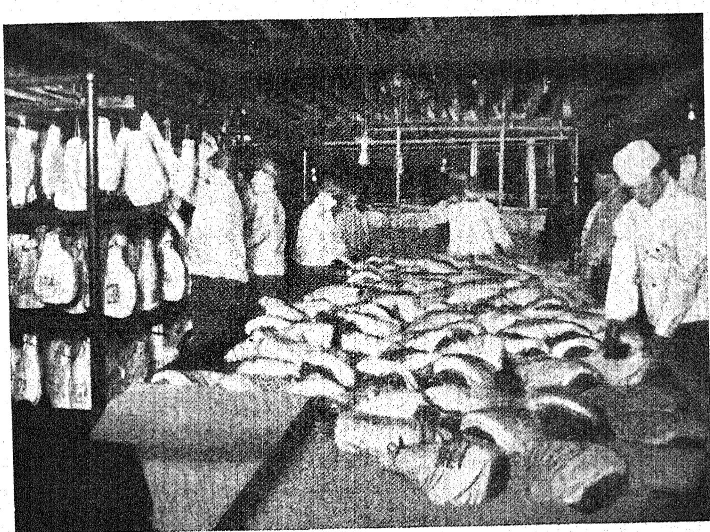


Fig. 100.—Preparing hams for smoking. (*Courtesy, Swift and Company.*)

posed of lactic acid bacteria and yeasts. These break down the tissues of the cabbage with the production of various acids. Properly prepared sauerkraut is light in color with a brittle texture; occasionally it develops a pink color due to the development within it of pink bacteria. This condition seems to be favored by excessive quantities of salt.

Preservation by the Use of Salt.—Beef, pork, fish, and many vegetables such as cucumbers, cabbage, cauliflowers, beans, and onions are readily preserved by salting. Meats may be cured by either dry salting or in brine. Vegetables are placed in large tubs or tanks and covered with a brine containing from 12 to 25

per cent of salt. The salt does not kill all micro-organisms, but the putrefiers and pathogens are retarded and in time probably destroyed. Salt tolerant organisms sometimes invade the tissues of meat and fish, discoloring, and rendering them unfit for use. *Cl. botulinum* does not grow in brine containing over 10 per cent of salt. Often the surface of the liquid is covered with an oil to prevent the growth of molds and other aerobic micro-organisms. Meats are usually dried to remove the excess of moisture on the surface in which micro-organisms would multiply. They are then smoked or various chemicals rubbed on the surface. These act as preservatives and prevent the micro-organisms which may reach the meat from gaining entrance.

Sugar.—Fruits are often preserved by the use of large quantities of sugar which makes the osmotic pressure of the substances so great that bacteria cannot multiply in them. Molds and yeasts are more resistant to osmotic changes, and are more likely to multiply in preserves than are bacteria. The molds are all aerobes; consequently, they do not multiply in the sealed containers. Grape sugar and levulose are more effective than is an equal weight of cane sugar. Salt and sugar are valuable means of preserving food as the salt can readily be removed by soaking, and the addition of water to the sugar-preserved product returns the product to its normal digestibility, if not to its normal appearance and flavor.

Chemical Preservatives.—It is generally agreed that chemicals which act by concentration are not objectionable, whereas others may be. It is quite generally recognized that formaldehyde and many other chemical preservatives are injurious. The Federal Pure Food Law prohibits these from being placed in food which is to enter into interstate commerce. Authorities disagree on benzoic acid, benzoate of soda, and several other chemicals, some claiming that they are injurious, others that they are not. However, in the case of some food preservatives the Federal Food Law requires that if these substances have been added they must be listed on the label. This permits the consumer to judge for himself whether or not he cares to use foods preserved by them. It is worth remembering that these chemicals preserve by inhibiting multiplication and the action of the digestive enzymes of the bacteria, because they are actually protoplasmic poisons. Substances poisonous to bacteria are usually poisonous to man and the enzymes used by bacteria in their digestive processes are similar to those used by man;

hence, if bacterial cells are to be injured or their enzymes rendered inactive by poisons, such preservatives will have similar action on human tissues and digestive juices. Moreover, chemical preservatives are especially vicious in the hands of unscrupulous manufacturers, for they make possible the use of products that could not otherwise be handled. They also make possible the handling of food by unsanitary methods.

CHAPTER XXXI

BACTERIA IN OTHER ARTS AND INDUSTRIES

Microbes have charge of life and death, of constructive and destructive changes. Some of these organisms are man's most destructive masters; others his most useful servants. Many are vital links in the chain which turns the wheels of life. They tear down the highly complex bodies of plants and animals from which the spark of life has departed and salvage the parts so they may be used again. They disintegrate waste products and change them into utilizable materials. Some microbes cause food spoilage often with disastrous results, whereas others are associated with the development of food flavors and at times even play a major rôle in food preservation. They manufacture alcohol, glycerol, acetic, butyric and lactic acids in addition to many other valuable products. They play a major part in the making of leather, the retting of flax, and in the curing of tobacco. In many changes they are the master chemists which initiate and control the reaction. Some of these processes have been known, although not understood, for ages; others are recent discoveries. Just a few of the most important ones can be considered in this chapter.

Silage.—There are approximately 500,000 silos in the United States, and the silage produced yearly runs into millions of tons. Any farm product can be siloed provided it contains sufficient sugar to produce the requisite acid for its preservation. Although corn is more extensively used than other products, even sunflowers and weeds have been used and make an acceptable and nutritious food. The product is cut into small pieces and tightly packed into the silo. If it is too dry, water is added. On the surface of plants are millions of bacteria, the number varying with the material used. Minimum and maximum counts as reported by Löhnis are:

Green forage per gram	2,000,000 to 200,000,000
Hay per gram	7,000,000 to 17,000,000
Straw per gram	10,000,000 to 400,000,000
Grain (cereals) per gram	100,000 to 12,000,000
Concentrated feed oil cake, etc.	10,000 to 20,000,000

The living succulent plant tissues contain many active enzymes. While the plant is growing the micro-organisms have no effect upon its tissues, and the enzymes are active in building new and repairing old tissues; but when the plant is chopped and packed into the silo the cell sap oozes from the injured parts. This mingles with the product and furnishes an excellent pabulum for the growth of micro-organisms. The enzymes of the dying tissues which during the growth of the plant systematically built up the tissues now commences to tear down. Within a few hours the oxygen is all consumed and the carbon dioxide con-

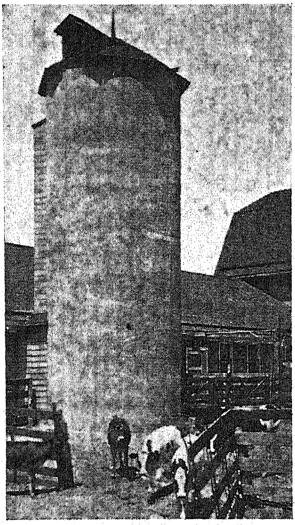


Fig. 101.—Silo built of reinforced cement. (*Utah Experiment Station.*)

tent is changed from 0.03 to 18 per cent. After the oxygen is all used anerobic changes occur, until by the end of the second or third day from 70 to 80 per cent of the silo gases are carbon dioxide. Occasionally a farm hand, ignorant of the gas, has been found dead in the silo, death having been caused by the lack of oxygen and not from a poisonous action of carbon dioxide. The changes which have occurred in the silage suppress some but favor other micro-organisms. There is not sufficient oxygen to meet the requirements of the molds. The acidity retards the action of the yeast and putrefiers. Hence, acid producers rapidly multiply and in a few days each gram of the silage may

contain 2,000,000,000 micro-organisms. Sherman found from 1,500,000,000 to 4,800,000,000 micro-organisms in each cubic centimeter of the silage juice Most of these were slender rods and he considered them to be nearly related to the *Lactobacillus bulgaricus* and *Lactobacillus acidophilus* groups.

The number of bacteria present reaches a maximum and then declines, and by the end of three or four weeks relatively few living micro-organisms remain. The predominating types which do remain are the streptococci and lactobacilli. Fermentation has almost ceased by the end of a month. The bacteria have done their work, and the enzymes have ceased to function. Heat is no longer generated but is slowly radiated from the silage. Acids, alcohols, and carbon dioxide have been produced from the sugars. The plant tissues have been broken down and the entire mass has changed in appearance, flavor, and nutritive value. The material has been preserved and will keep for months or even years.

The changes which have occurred are great. From 10 to 15 per cent of the total dry matter has been lost. Most of this has gone off as carbon dioxide. Some may have been lost in the plant juices which occasionally drain from the silo. Much of the sugar has been transformed into acids. The quantity varies with the silage as may be seen from the following results reported by Neidig:

Silage from	Percentage acid in silage			
	Acetic acid	Propionic acid	Butyric acid	Lactic acid
Corn	1.8–4.0	0.2–0.3	0.0	4.0–6.1
Sunflowers	1.1–3.4	0.0–0.4	0.0–3.1	1.5–3.5
Oats and peas	1.5–2.2	0.1–0.2	0.0	4.6–5.0
Clover and straw	2.0–2.5	0.2	0.0	2.8–2.9
Alfalfa and straw	1.4–2.0	0.2–1.0	1.2–2.2	0.0

Alcohol is produced to the extent of about one fourth of 1 per cent. The proteins are partly hydrolyzed. There is a decrease in the crude fiber. The ether extract increases, yet it is doubtful if there is any increase in fat. The green coloring material, chlorophyll, is changed to a brownish pigment.

The changes which occur in silage and the nature of the final product varies with the material siloed, the nature of the silo, and the temperature. Gorini distinguished four types of grass silage prepared in pits, depending upon the predominating type of bacteria. They were butyric, lactic, putrefactive, and sterile or atypical. Butyric is likely to predominate where beet tops are siloed.

In heavily soiled material putrefactive and butyric acid changes usually predominate, and the resulting silage is very inferior or it may be a complete loss. It may also at times be dangerous. It is especially objectionable because of the odor and taste which it is likely to impart to the milk. Moreover, the bacteria which enter from the surroundings render the milk unsuitable for cheese making. Lactic acid predominates in silage properly made from corn and other products having a sufficient quantity of readily fermentable sugar. It is the ideal product.

Putrefactive changes predominate in silage made from materials high in proteins but low in sugars especially if carrying soil. If the silage stage reaches a temperature of 60° C. butyric acid organisms predominate; if 50° C., lactic acid organisms prevail; putrefaction occurs at lower temperatures, and sterilization when the mass becomes superheated. Attempts have been made in Switzerland to control the temperature by electrical means. The results are promising in so far as the quality of the product is concerned, but whether it warrants the extra cost remains to be seen.

Vinegar Making.—There are two steps in vinegar making, each brought about under definite conditions by specific microorganisms. The first is the production of alcohol by microorganisms, usually yeast acting on a sugar. Any material which contains from 12 to 16 per cent of sugar, such as fruit juice, malt extract, diluted honey, or molasses, is suitable raw material for alcohol production. Although alcoholic fermentation usually will occur due to organisms which are on the product, the best results are obtained by seeding with a suitable culture of yeast. If the alcoholic liquor is produced from fruit or grain extracts which have been decomposed by foreign bacteria, neither the alcoholic fermentation nor the acetic acid fermentation is normal; hence, the resulting vinegar has off flavors. The rate of the reaction varies with the temperature. The optimum lies between 22° and 26° C. The time required for the completion of the alcoholic fermentation in the ordinary farm process where

the freshly pressed fruit juice is placed in casks and allowed to stand at a temperature of from 7° to 13° C. is six months. Alcoholic fermentation should be complete before the starting of the acetic acid fermentation as yeasts do not work well in the presence of acetic acid.

The second step in the manufacture of vinegar is an oxidation process. It is brought about by the action of the acetic acid bacteria upon the alcohol which is caused to take up oxygen and becomes acetic acid. Ordinarily, the vinegar bacteria if undisturbed form a film on the surface of the alcoholic solution. This is known as the "mother of vinegar." When Pasteur investigated the troubles of the vinegar makers of Orleans, he found that they did not appreciate the great importance of supplying their fermenting mixtures with plenty of oxygen. In order to provide this, he made more openings in the casks and devised a raft of slats. This was placed on the surface of the liquor and acted as a support for the mother of vinegar.

There are two methods in general use for the manufacture of vinegar from alcoholic liquors: The Orleans, and the quick or German methods.

The Orleans Method.—This is the oldest commercial method and produces vinegar of the highest quality. However, it has the disadvantage of being cumbersome, laborious, slow, and costly. Then too, there is a loss of about 10 per cent of the material by evaporation, and the repeated additions of liquid break the bacterial film which sinks to the bottom and grows anaerobically exhausting the nutrients without the production of acetic acid. There are many modifications of the method, but they are all based essentially on the same principles. The filtered wine is placed in barrels or covered vats furnished with openings so that the entrance of air is facilitated and can be controlled. The receptacle is filled about two-thirds full with a mixture of four parts of good, new vinegar, and six parts of wine, preferably that which has been pasteurized at 55° C. A small quantity of a good bacterial film is put into this solution as a starter. Periodically a portion of the contents is drawn off and replaced by wine and so the process continues. Pasteur changed to shallow vats with regulated air vents instead of barrels or vats, and on the top of the fermenting liquor he placed a perforated wooden float to support the "mother of vinegar." Acetic acid bacteria will grow slowly in a weakly acid or even slightly alkaline medium, but a more prompt and vigorous growth is obtained in a decidedly acid medium. For this reason, vine-

gar is added to speed up the action of the acetic acid bacteria and to prevent the growth of undesirable micro-organisms.

The Quick Vinegar Process.—In this method large wooden tanks with perforated bottoms are used. These are filled with beech chips, the pressed pomace of red wines, rattan shavings, corn cobs, or charcoal. Although the main function of these is to increase aeration, the best results are obtained with beech shavings, or pomace, for they impart no undesirable flavor or color to the product. The solution to be acidified is automatic-

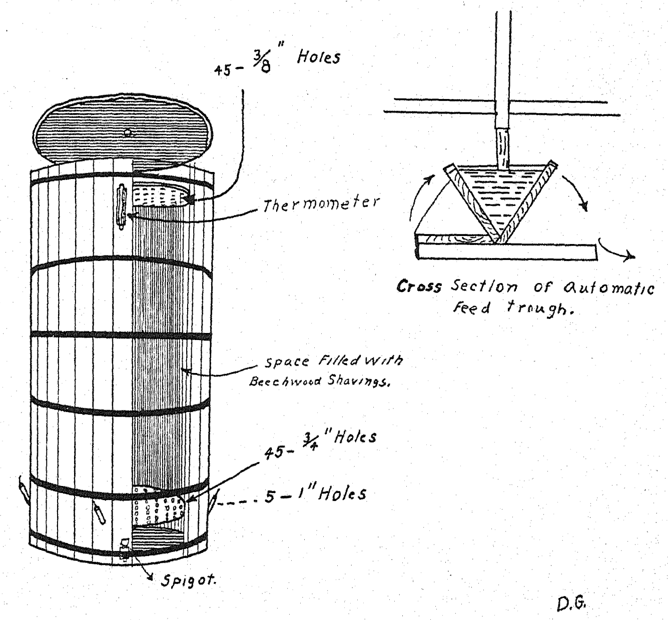


Fig. 102.—Drawing of barrel used in vinegar making.

ally sprayed on the surface of the shavings and slowly trickles down in a continuous film. This is acted upon by the acetic bacteria that have previously been seeded on to the shavings, and the heat generated causes a constant draft which furnishes the micro-organisms with the requisite oxygen.

On standing for some time vinegar may lose its strength because of the action of certain bacteria (*Acetobacter xylinum*) which in the presence of oxygen split the acetic acid into simpler compounds. To avoid this, the finished product is pasteurized or stored in air-tight containers. Pasteurization is believed by

some to assist in the maturing of vinegar, giving to it a softer and more pleasant taste and aroma. This is thought to be due to the hastening of the reaction of the residual alcohol and the acetic acid with the formation of esters.

Curing of Tobacco.—Tobacco undergoes profound changes during the curing period. The texture and aroma are greatly influenced. There is about a 28 per cent loss of nicotine and a gain in citric acid. These are fermentative changes caused by enzymes and micro-organisms. Some investigators have made studies in the laboratory in which they have used pure cultures and obtained what they considered to be a superior product with a more satisfactory aroma than is obtained when the tobacco ripens under natural conditions. The bacteria found in the curing of tobacco seem for the most part to belong to the proteus, subtilis, and mycoides groups. Other investigators have obtained equally good results in curing tobacco in the presence of disinfectants which prevented the actions of micro-organisms but did not interfere with enzymic action. Others have found that as the temperature of the fermenting tobacco increases, it gradually becomes sterile. Loew holds the idea that the curing of tobacco under natural conditions is not essentially a bacterial process but due to leaf enzymes, oxidases, peroxidases, and catalases. Probably both enzymes and micro-organisms play a part, but at the present time it is impossible to state which is the more important. A British patent has been taken out for the improving of inferior or ordinary tobacco and giving to it a flavor resembling Havana and other superior qualities. The material is treated with liquid cultures of one or more of the following organisms: Butyric ferments, aromatic lactic acid bacteria, ester-forming species of torula, and ester-forming fungi. The cultures may be grown in various liquids such as malt extract, milk whey, and solutions of different sugars along with decoctions of various aromatic vegetables, and this applied to the tobacco or the cultures; or the unfermented material may be added and the fermentation permitted to occur on the tobacco.

Cocoa.—Cocoa and the various chocolate and cocoa preparations are made from the beans of the cocoa tree, *Theobroma cacao.* The beans grow in pods, varying in length from 23 to 50 cm., and are from 10 to 15 cm. in diameter. The beans which are about the size of almonds are closely packed in the pod, and are white in color; upon drying they turn brown. These beans are mixed with plantain leaves and placed in barrels. The sweet outer covering of the bean immediately commences to

ferment and the mucous covering is liquefied. Heat develops due to the fermentation. After about four to seven days the seeds are taken out, spread on trays, covered, and left for another day to complete the process. Loew says: "The process of fermentation depends especially on yeast cells which increase rapidly in the syrup of the cocoa bean, with the formation of alcohol and carbon dioxide. Also bacteria take part in the fermentation, oxidizing the alcohol to acetic acid. A rise in temperature takes place and the slimy tissue surrounding the seeds comes off and collects on the bottom of the vat. The removal of this tissue is the chief end of the fermentation. It causes the seeds to be more easily dried. An oxidation of the tannin takes place, which causes the brown color of the bean. The taste and the aroma depend on the fermentation as well as on the roasting."

Retting of Flax and Hemp.—"Hemp was probably the earliest plant cultivated for the production of a textile fiber. The 'Lushi,' a Chinese work of the Sing dynasty, about 500 A. D., contains a statement that the Emperor Shen Mung in the twenty-eighth century B. C. first taught the people of China to cultivate 'Ma' (Hemp) for making hempen cloth." Hemp makes a coarser fabric than does flax, but the fiber from both plants is separated by a process of retting or rotting. This may be carried out either by dew retting or water retting. "In this country dew-retting is practiced almost exclusively. The hemp is spread on the ground in thin, even rows, so that the butts are all even in one direction and the layer is not more than three stocks in thickness. Warm, moist weather promotes the retting process, and cold or dry retards it. Hemp rets rapidly if spread during early fall, provided there are rains, but it is likely to be less uniform than if retted during the colder months. It should not be spread early enough to be exposed to the sun in hot, dry weather. Alternate freezing and thawing or light snows melting on the hemp gives most desirable results in retting. Slender stocks one-fourth inch in diameter or less ret more slowly than coarse stalks. The finer stalks are usually not over-retted if left on the ground all winter. Hemp rets well in young wheat or rye, which holds the moisture about the stalks. In Kentucky most of the hemp is spread during December. A protracted January thaw with comparatively warm rainy weather occasionally results in over-retting. While this does not destroy the crop, it weakens the fiber and causes much loss. When retted sufficiently, so that the fiber can be easily separated from the

hurds or woody portion, the stalks are raked up and set up in shocks, care being exercised to keep them straight and with the butts even. Water-retting is practiced in Italy, France, Belgium, Germany, Japan, and China, and in some localities in Russia. It consists in immersing the hemp stocks in water in streams, ponds, or artificial tanks. In Italy where the whitest and softest hemp fiber is produced, the stalks are placed in tanks of soft water for a few days, then taken out and dried, and returned to the tanks for a second retting. Usually the stocks remain in the water first about eight days and the second time a little longer.

"In either dew-retting or water-retting the process is complete when the bark, including the fiber, readily separates from the stalks. The solution of the gums is accomplished chiefly by certain bacteria. If the retting process is allowed to go too far, other bacteria attack the fiber. The development of these different bacteria depends to a large extent upon the temperature. Processes have been devised for placing pure cultures of specific bacteria in the retting tanks and then keeping the temperature and air supply at the best for their development. These methods which seem to give promise of success have not been adopted in commercial work."

The fermentation process consists essentially in dissolving the cementing substance known as pectins which hold the bast fibers to the stock. This is due to pectinase which is produced by a number of micro-organisms. Two active pectinase-secreting bacteria which play a part in the retting of flax are *Bacillus filsinens* and *Clostridium butyricum*.

In Belgium and Holland where much of the finest retting is done, it is stated that certain streams are better adapted to the retting process than are others. This may be due to a variation of the microflora of the various streams, as it is likely that some organisms are better adapted to carry on the process than are others.

Efforts have been made to liberate the flax fiber by means of chemicals, but none of these efforts has been fully successful as it is found that chemicals strong enough to dissolve the pectin also weaken the flax fiber and destroy its luster. However when it becomes possible to produce pectinase on a commercial scale it will probably be used in the retting of the flax.

The Tanning of Hides.—The making of leather is an art, the history of which extends into antiquity. Many half-civilized people knew how to tan hides. The American Indian was an

expert in the making of leather from many hides. "The leather industry of today is an evolution covering many centuries. It is a far cry from the crude primitive methods employed in the earliest historic period to the highly complex process of the modern tannery. Probably the first method of curing skins was simply cleaning and drying. Then it was found that the use of smoke, sour milk, various oils, and even the brains of the animals from which the hides were stripped improved the quality and texture of the leather; still later it was discovered that certain astringents, barks, and vegetables effected permanent changes in the texture of the skins and stopped decay. The use of bark and vegetable juices has continued and forms the basis of many of the modern tanning processes. The Egyptians possessed this knowledge, for pictures and hieroglyphics describing the methods are found on many of the ancient tombs in the Nile Valley. Three thousand years ago the Chinese were familiar with leather preservation, as various relics handed down from antiquity serve to show. Leather preserved and tanned with oil, alum, and bark was also used by the Romans."

The various steps which enter into the making of leather from hides may be grouped under the several heads: (1) The preservation of the hides; (2) disinfection; (3) the soaking and fleshing; (4) depilation; (5) batting; and (6) the final tanning process. The transformation which occurs in many of these processes, especially in the early methods of tanning, is brought about by microbial action.

Although the inner surface of the skin as it comes from the animal is free from bacteria, it rapidly gathers them from various sources. These if permitted would multiply rapidly and under appropriate conditions destroy the skin. To prevent this the green hide is treated with salt; this reduces the water content of the skin from 65 to 45 per cent. It also stops the action of bacteria on the proteins of the skin and at low temperatures as long as the wetting solution contains 6 per cent salt, there is little danger of putrefaction. If the strength of the salt solutions falls below 1 per cent it favors putrefaction. The process of salting is known as curing.

Because infectious diseases have been spread by infected hides it is the custom to disinfect some hides. This is done by soaking them in a 2 per cent solution of mercuric chloride containing 1 per cent formic acid, or a 10 per cent solution of salt in 2 per cent hydrochloric acid. This is especially necessary in the case of hides which have come from animals infected with *B.*

anthracis which is pathogenic to man and may survive the various tanning processes.

On reaching the tannery, the hides are first soaked to render them pliable and to permit bacteria to remove the excess tissue which adheres to them. In the old process bacteria were depended upon to remove most of the excess flesh, but today this is accomplished primarily by passing the skin over revolving rollers on which are knives which cut away the excess tissue. Bacterial counts are occasionally made of the soak water so as to guard against excessive numbers.

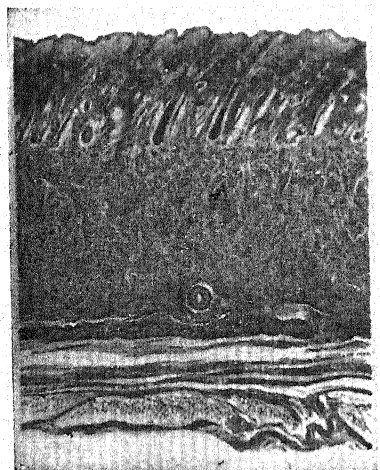


Fig. 103.—Vertical section of calf skin (fresh) magnified 30 diameters. (*Courtesy, A. F. Gallun and Sons Company.*)

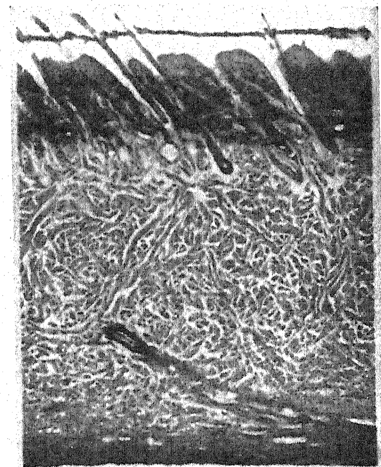


Fig. 104.—Vertical section of calf skin (after forty-eight hours in lime liquor) magnified 35 diameters. (*Courtesy, A. F. Gallun and Sons Company.*)

Following the soaking comes the depilation—the removal of the hair. This is accomplished in a number of different ways such as liming, sweating, use of enzymes, acids, or alkalis. The earliest method of removing the hair from hides was by means of incipient putrefaction. This causes the soft mucous matter of the epidermis to become altered, and the hair to become loosened without materially injuring the true skin. This method is still employed by many manufacturers of sole leather and is called sweating. The operation is conducted in closed rooms which are kept at a temperature of 70° F. The hides are hung in small chambers ("sweat pits") holding about 100 hides

each. A large quantity of ammonia is given off during the decomposition which aids in the solution of the epidermis and the loosening of the hair. After four to six days of this treatment the hair is sufficiently loosened to be removed by working over a rounded beam with a blunt knife made for the purpose. Hides which have been unhaired in this way are soft and fallen and must first be swollen by placing in an acid liquor before entering the tanyard. This treatment is especially necessary in the case of sole leather. However carefully this operation is conducted, there is liability that putrefaction may attack the skin itself, thus causing a weak grain. For this reason this method has been to a great extent replaced by the liming process. The hides are soaked for some time in a saturated lime solution. The lime causes the hide to swell, softens the epidermal cells, dissolves the mucous layer, and loosens the hair so that on scraping with a blunt knife, both the epidermis and hair are easily removed. The solution increases in efficiency with use. This is thought to be due to the bacteria which accumulate, as the medium soon contains thousands of proteolytic ferments. Wood has isolated 90 species of bacteria from such a vat, none of which possesses the power of bringing about the desired change, but all acting conjointly produce the desired product.

The lime is removed and the material is brought from its swollen to a soft and open condition by placing in a weak fermenting infusion of pigeon or hen manure. The acids remove the lime salts. The process is called batting. The product is next transferred to the tan pile or into vats containing the tan liquors. In some of these processes micro-organisms play a major rôle in finishing the product.

Microbial Production of Organic Compounds.—It has been known for a long time that micro-organisms produce many organic compounds of commercial value but it is only within recent years that attempts have been made to produce them on a commercial scale. Citric acid, gluconic acid and glycerin may be taken as examples.

Citric Acid.—Citric acid occurs in the citrus fruits and is of considerable commercial importance and in some places the growing demand is coming to exceed the supply from this source. Consequently, interest in other sources has been awakened. Wehmer in 1892 described an interesting group of Penicillium-like fungi, a number of which produce large quantities of citric acid when grown on carbohydrates in the presence of suitable

nitrogenous and mineral materials. To these he gave the name *citromyces*. Two species *Citromyces pfeifferianus* and *C. glaber* fermented as high as 50 per cent of the glucose with the production of 8 per cent of citric acid. Even greater yields have been obtained when chalk is used to neutralize the acid as formed. Several patents have been taken out in the United States and Germany in which these and other micro-organisms are used to ferment molasses and other by-products rich in carbohydrates. A recent one in which the *p*H is adjusted to 1.2 to 1.5 with hydrochloric acid gives yields of citric acid up to 65 per cent of the sugar decomposed. So far, the method has

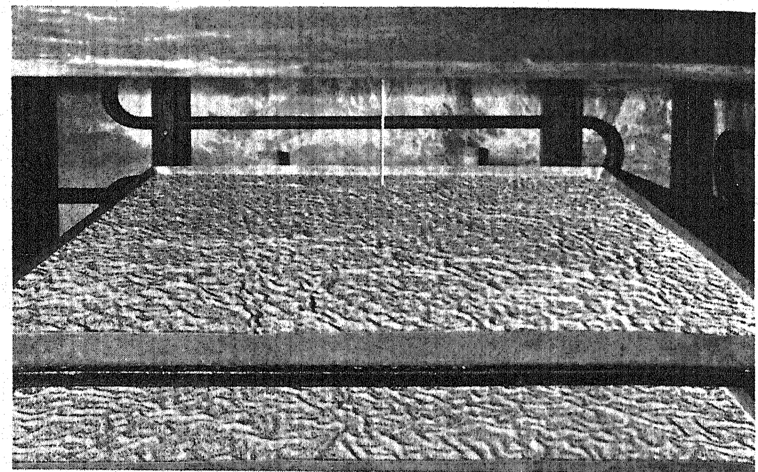


Fig. 105.—Typical pan fermentation in the manufacture of gluconic acid by *Penicillium luteum purpurogenum*. (*Courtesy of O. E. May.*)

failed to actively compete with the acid prepared from the fruit but with increasing demands it is promising.

Gluconic Acid.—One of the newest and most promising fermentations suitable for industrial use is the production of gluconic acid from glucose. May and coworkers have produced it on a semiplant scale by the action of *Penicillium luteum purpurogenum* on solutions of commercial glucose. They used seven aluminum pans 43 × 43 × 2 inches and produced 36 Kg. of gluconic acid in an eleven-day run, the yield corresponding to slightly over 57 per cent of the theoretical. The acid is precipitated as calcium gluconate which is used therapeutically to increase the calcium content of the blood. The cost of manu-

facturing is approximately 90 cents a pound which is considerably below the straight chemical methods.

Glycerin.—Glycerin, since its discovery in 1779 by Scheele, has attracted special attention. This has been due to its high boiling point, its softening effect on the skin, and value in cosmetic preparations, its miscibility with alcohol and water, its great solvent powers, its sweet and agreeable taste, and finally its value as a base in the preparation of nitroglycerin and dynamite.

The world output, cost, and use varies widely from time to time. It is a by-product in the manufacture of soap and candles, consequently, its output depends upon the demand for these products. The demand for glycerin depends upon war and peaceful pursuits. During the World War it became very expensive and the increasing uses in the peaceful pursuits tends to keep the price at a high level.

Pasteur recognized that glycerin is a by-product in alcoholic fermentation and for years, about 3 per cent of glycerin has been obtained from this source. The demand for glycerin during the World War increased interest in this fermentation and resulted in the establishment of factories in which glycerin was the main and not the by-product. There were developed two processes, the alkaline process and the sulfite process.

In the alkaline process some cheap product, for example black strap molasses, is fermented with special strains of *Saccharomyces ellipsoideus*. The temperature is kept at approximately 30° C. As the fermentation proceeds, sodium carbonate together with fresh portions of the carbohydrate-carrying material, are added. Yields as high as 25 per cent of the added sugar have been reported. The alkaline condition tends to increase infection with lactobacilli which lessen the yield. Consequently, there is a tendency to prefer the sodium sulfite process. In this process, yields as high as 36 per cent have been reported. All kinds of fermentable sugars can be used as can also all actively fermenting yeast strains. The yeasts have to be educated to ferment in the presence of high concentrations of sodium sulfite. Consequently, they must be added by degrees. It is stated that this method can compete with the production of glycerin from fats, but this will vary with the demand for glycerin and the cost and availability of the raw materials.

CHAPTER XXXII

AIR

COMMON air is at the basis of our very existence. We obtain our oxygen supply directly from the air and indirectly through the plants, carbon, hydrogen, and nitrogen. Air controls the light, temperature, and moisture of our surroundings and brings to the plants their required food. We could live for weeks without food and for days without water, but only a few moments without air. Air is the cheapest, most abundant, and most essential of the three. Everyone desires a pure water; some give considerable attention to the quality and quantity of the food they consume, but many are quite indifferent as to the nature of the air they breathe.

Composition.—The atmosphere at or near the surface of the earth is composed of 78.1 per cent by volume of nitrogen, 20.9 per cent by volume of oxygen, and 0.9 per cent by volume of argon. Carbon dioxide is normally present to the extent of 0.03 per cent, and the inert gases other than argon (neon, helium, xenon, krypton) in still smaller percentages. Air also carries varying quantities of moisture, dust, soot and micro-organisms.

Micro-organisms in Air.—For normal growth and reproduction all bacteria require water, food, a suitable temperature, and usually the absence of direct sunlight. The moisture conditions of the atmosphere at times may be optimum, but generally none of the other conditions are; hence, soil, water, and milk have a natural microflora, but air has not.

The micro-organisms in the atmosphere vary from place to place and from day to day. They enter the air from the surroundings. The bacterial content of dust varies from a few thousand to many million in each gram. The hurrying feet, rapidly moving wheels, and the ever-acting currents of air carry them into the atmosphere. The spray from the beating surf, the droplets from the mouth of the speaking, laughing multitudes, all add their quota—in short—anything which increases the suspended particles in the atmosphere usually increases its microbial content. The exception is where antiseptic substances are finding their way into the air. For example, the air of Lon-

don streets contains between 300,000 and 500,000 solid particles per cubic centimeter, but there is only one micro-organism to every 38,200,000 dust particles.

Bacteria are most plentiful in the air of densely populated areas. They are especially numerous in some factories, churches, theaters, dance halls, and other places where large numbers congregate. Miquel found that on an average 1 cubic meter of air from the streets of Paris contained 3480 bacteria; laboratory air, 7420; and the air of old houses 79,000. Winslow and Browne found 1 foot of air from the country to contain 56 microbes; city street air, 72; office and school air, 95; and factory air 113. They found streptococci (characteristic of specific mouth pollutions) in outdoor air to the number of 11 to 12 per 100 cubic feet; in office air, 22; school air, 30; and in factory air, to the number of 43. The number of micro-organisms in the air also varies with the distance from the earth at which the air is collected. At the top of the clock tower of the House of Parliament in London Graham Smith found only one third the number of bacteria that he found at ground level. Whipple found 1330 bacteria per cubic foot in air at street level, whereas at the tenth story of the John Hancock Building the air contained only 330. Furthermore, the classic experiments of Pasteur showed the air of streets and workshops to contain many micro-organisms, whereas the air over the surface of glaciers and on tops of high mountains was often free from them.

Factors Governing Number in Air.—Moisture greatly increases the number of bacteria in soil, but it decreases the number in the air, for it moistens the particles of dust, causes them to settle out and to carry with them the bacteria. Country air contains fewer bacteria than does the air of the city. This is due in a measure to the larger quantity of organic matter in the dust of the city, and also to the numerous ways in which bacteria are being carried into the air.

On mountain tops, over deserts, and in other uninhabited regions the air is nearly free from bacteria. The classic illustration of this fact is found in the experiments that Pasteur conducted in his efforts to refute the doctrine of spontaneous generation. Of twenty flasks exposed to normal country air at Arbois, eight showed the presence of micro-organisms, whereas a similar series of sterile flasks opened on the slopes of the lower Jura mountains only five spoiled; and of a third series exposed on the Mer de Glace, only one became infected.

The number of bacteria in air over oceans is low and varies

with the nearness to land. Close to shore there are often very many, whereas at great distances from land the air may be free from micro-organisms.

The number of micro-organisms in air varies with the locality and decreases with the altitude. Jean Binet did not find a single microbe in 100 liters of air taken on the summit of Mount Blanc. The number rapidly increased on descending.

At the Summit	0
At the Grand Plateau	6
At the Grand Malet	8
At the Place de l'Aiguille	14
At the Mer de Glace	23
At Montanvert	49

The numbers vary with the season, increasing from winter to summer and decreasing from summer to winter. There is also a marked decrease in the number of bacteria in the air after a rainstorm. The rain carries them to the ground and also moistens the surface so that particles of dust are not carried into the air by every breeze. However, the added moisture of the soil greatly increases the speed of multiplication, so that later as the surface soil dries out, more dust and with it a greater number of bacteria are carried into the air. It is also true that the number of micro-organisms in the air decrease in the winter months not because cold is inimical to the life of the micro-organisms, for just the reverse is true, but because the conditions are not so good for them to find their way into the atmosphere. This is due to the soil being covered with snow or with considerable moisture which prevents the dust from being carried into the atmosphere.

It is quite evident that there would be a relationship between the number of bacteria in the atmosphere and the climate of that region. Bacteria multiply rapidly in the soils of a warm, humid district and find their way into the atmosphere where they remain until they are washed out by rain. There is, as one can readily see, a great variation in the bacterial content of the air of warm moist regions. In arid places the number in the air may be smaller but does not vary as greatly as in the more humid sections.

Bacteria remain in the atmosphere for varying lengths of time. Their stay depends on several factors:

1. The hardy spore-forming saprophytes may remain suspended in the air for days or even weeks, whereas the frail non-

spore-forming pathogens soon perish due to either drying or the sterilizing action of the sun's rays.

2. Small particles settle out more slowly than do large ones, for as the size of an object is decreased the surface area decreases less rapidly proportionately than does the volume. Hence, those bacteria which are floating free in the atmosphere settle out more slowly than those attached to dust particles.

3. The time of suspension is also determined by the velocity of the air current. Micro-organisms settle out of a still atmosphere more readily than from one in motion. It may require an air current of considerable velocity to dislodge micro-organisms and bring them into suspension, yet a slight current will sustain them.

4. Moisture in the atmosphere tends to cause particles to adhere together, and as they grow in size the tendency for them to settle out is proportionately increased.

5. Although the air of London and many other large cities contains numerous particles of dust, the number of living organisms is comparatively small, for the various gases thrown into the atmosphere have a slight germicidal effect upon the bacteria.

Bacteria in Inspired and Expired Air.—The number of bacteria inhaled by man depends on the number of micro-organisms in the air. It is estimated that a person living in London breathes about 300,000 bacteria each day, and individuals living in other districts may take in many times this number. Most of these bacteria are harmless and are caught on the moist mucous membranes of the upper respiratory passages, only very few finding their way into the deeper alveoli, and the majority of those that do are quickly destroyed by the phagocytic cells of the body.

It has been shown that the expired air is entirely free from germs. The reason for this is that they are not thrown off from moist surfaces. Even a considerable current of air fails to dislodge them. The expired breath of an individual suffering from diphtheria, tuberculosis, or any of the other respiratory diseases is entirely harmless. Coughing, sneezing, or forceful speaking may dislodge them and cause them to be thrown off in a fine spray, but this does not occur in normal, or even deep breathing.

Droplet Transference of Bacteria.—Flügge was the earliest investigator to call attention to the fact that during speaking, and especially during loud talking, coughing, and sneezing, tiny droplets of saliva are thrown into the air. Anyone can observe the fine spray coming from the mouth of individuals standing

in certain lighted positions during these actions. In order to test the extent to which this spray may carry bacteria into the air, various investigators infected their own mouths with *Serratia marcescens* and then read, spoke, coughed, and sneezed in rooms in which cultural dishes were exposed. These dishes collected and indicated the presence of the organism. In some cases, it was found that the germ-laden droplets expelled during coughing were carried to a distance of 9 meters from the mouth, and in a few cases to the distance of 2 meters behind the person coughing. The comparative results as obtained by various investigators are tabulated below.

	Total plates exposed	*Serratia marcescens* figures indicate colonies per person	Percentage of positive plates
Laschtschenko	36	17.2	83
Koeniger	331	5.2	61
Gordon	30	0.7	53
Winslow and Robinson	325	2.4	48

Some have interpreted these results as indicating that a general infection of the atmosphere takes place over a wide radius and that there is a real danger from breathing the air in the neighborhood of an individual ill of any of the respiratory diseases. This is more apparent than real, for Koeniger showed that 60 per cent of the bacteria disappear from the air in ten minutes, while after twenty minutes less than 10 per cent of the original number remain.

Where attempts were made after speaking, coughing, etc., to detect organisms in the air which are normal inhabitants of the mouth it became evident that the results obtained in the artificial infecting of the mouth were far too high. Quantitative tests of the air itself at 35 cm. to 2.4 meters in front of speakers who spoke vigorously in English or German, usually failed to reveal mouth streptococci in large volumes of air. The result is that Flügge's own treatment of the matter is considered conservative by most health workers today. He pointed out that in experiments carried out by his own pupils, tubercle bacilli were abundant only within 0.5 meter of the coughing patient and that beyond 1.5 meters their numbers were so small as to make the chances of infection practically nil. The rule laid down by him is the best yet formulated for the prevention of the spread of the

respiratory diseases: "During strong paroxysms of coughing, the consumptive should keep at arm's length from his companions and should hold a handkerchief before his mouth. In work rooms, offices, and such places the space between the workers should be at least 1 meter."

Air-borne Infection.—The theory that miasmatic vapors in the atmosphere are the cause of disease dates back to the times of Hippocrates when fires were lighted in the streets of Athens to purify the air and put a stop to the plague. Such a belief was only natural, for the viruses of the communicable diseases were believed to be gaseous, or at least readily diffusible substances borne by air currents. And what was more reasonable than to believe that these entered the air from the breath of the patient coming as the breath does from the very vitals? Night air was considered to be especially bad, for it was believed to carry malaria to the individual. The formulating of the germ theory of disease strengthened this belief in the minds of many. Germs of disease are often found in the nose and throat of the sick, and it seemed reasonable to believe that such tiny particles easily could find their way into the air where they might float for some time. The work of Pasteur demonstrated that in so far as the decomposition of organic material was concerned the germs carried by air play a predominant rôle. When Lister in 1867 devised his first antiseptic spray, it was the microbes of the air which loomed largest in his mind as potent factors in wound infection.

The first shock to this belief came when it was proved by experiments that the expired air is sterile. Then too, the careful quantitative work of Winslow and others showed that pathogens forced from the mouth and nose in the acts of speaking, coughing, and sneezing travel only short distances in the droplet, and that when dislodged from the droplet soon perished because of the action of the oxygen of the air, light, and the loss of moisture. Of the numerous careful epidemiological studies that have been made of various diseases, all have yielded negative results. The work of the surgeon has clearly demonstrated that even the widely distributed pyogenic organisms get into the wounds not from the air but from the skin of the patient, or the instruments which produced the wound, or other objects which came in contact with it. Moreover, it is the experience of well-managed hospitals that various communicable diseases may be kept in a single open ward without serious danger of cross infection, provided care is taken to prevent contact infection. Even

the malaria, yellow fever, and the bubonic plague, which seemed to settle from the air on whole cities at a time, are now known to be conveyed by insects, and not by air; and it was the conclusion of the Influenza Commission after its study of the great influenza epidemic which so quickly spread over all quarters of the globe, that this disease, also, travels in a "pair of shoes" and not in the air. Hence, today it is the consensus of opinion among health authorities that this spectre of air infection, which has plagued the race from the times of Hippocrates, plays little if any part in the transference of disease. Today efforts are used to prevent contact infection and not air infection.

Odors and Health.—It has long been a popular belief that foul air not only causes illness but often gives rise to communicable diseases. Leading sanitarians taught from 1880 to 1890 that sewer gas gave rise to diphtheria and other diseases. The laws concerning nuisances as they exist today in many cities and states are based on this belief. Careful examination of the gases emitted from sewers and cesspools failed to reveal the pathogens. Today it is known that sewer air, unless there be a splashing and particles are mechanically thrown into the air, is as free of pathogens as is the east wind, and consequently cannot give rise to communicable diseases.

Coal gas, illuminating gas, chlorine, hydrogen sulphide, and other chemical gases may injure health, or even cause death as a result of their direct poisonous action. Lower animals as well as man do best under pleasant agreeable surroundings; hence, it is not surprising to find, as Winslow did, that guinea pigs exposed to the odor of putrefying organic material showed a less rapid growth curve for the first few days of exposure than normal animals. However, the effect was only temporary and the animals later caught up with the controls. The New York Commission on Ventilation found that offensive odors which came from improper ventilation and putrefaction had two slight but distinctly measurable effects upon human behavior. Subjects exposed to stale air showed a definite disinclination to physical activity and accomplished 9 per cent less work in stale than in fresh air. The effects also manifested themselves in the appetite of the individual, for the amount of food eaten at luncheon by those who were kept in fresh air was from 4 to 14 per cent more than that eaten by those kept in stale air. The subject is summarized by Winslow as follows: "We may, therefore, conclude that while air does not as a rule bear the germs of disease and does not carry with it mysterious and specific

organic poisons, an atmosphere laden with offensive odors is not only objectionable on esthetic and economic grounds, but may under certain circumstances constitute a real menace to the public health."

Ventilation.—Many changes occur in the air of an unventilated room as a result of human occupancy. These may be grouped under five heads: (1) The oxygen content of inhaled air is 20.9 per cent by volume, whereas that of exhaled air is only 16 per cent. A man of average weight, at rest, consumes about 534 liters of oxygen in twenty-four hours. (2) Inhaled air contains 0.03 per cent by volume of carbon dioxide and exhaled air 5 per cent. Under average conditions of rest an individual will produce about 427 liters of carbon dioxide in twenty-four hours. (3) There is given off into the air from the body in the breath, perspiration, and from the clothing a variable amount of organic substances which give to the air the "stuffy odor" which one notices on entering a poorly ventilated room. (4) The temperature of the air is raised due to oxidations occurring in the human body. As an average for each individual, the heat liberated amounts to approximately 2500 calories in twenty-four hours. (5) The humidity of the air is increased due to the moisture given off in the breath and perspiration. The amount is variable, but the contribution is about 1400 cc. in twenty-four hours. If the individuals are engaged in physical activity these quantities may be doubled, trebled, or even quadrupled.

In a crowded, ill-ventilated room one experiences a feeling of lassitude, dulness, sleepiness, and if continued long enough there results nausea and faintness. There are cases on record in which it has been carried to such an extent that death has resulted.

The effects have been attributed by various workers at different times to the following changes: (1) A loss of oxygen; (2) an increase in the carbon dioxide; (3) poisonous organic substances given off from the body; and (4) temperature and moisture changes which have occurred in the air.

The effect cannot be due to a diminution of the oxygen content, as even in the most poorly ventilated rooms oxygen never falls below 20 per cent, and it is only when it falls from its normal of 20.9 to 16 per cent or less that important physiologic reactions occur. Individuals often live at altitudes, and in reality some of them are known for their beneficial effects on health, where the oxygen pressure is far lower than it ever becomes in poorly ventilated rooms.

The ill effects of an atmosphere vitiated by human beings

cannot be attributed to an increase in the carbon dioxide content, for even in the most poorly ventilated rooms it is unusual to find 0.5 per cent carbon dioxide. The air of breweries has been found to contain from 1.5 to 2.5 per cent carbon dioxide, yet men have worked continuously in these without any ill effects. Moreover, numerous experiments have definitely proved that an atmosphere may carry as high as 3 per cent of carbon dioxide without producing any measurable ill effects upon human beings.

It is not probable that the ill effects are caused by a poison exhaled in the breath, for repeated search for such a substance has yielded only negative results. Hence, it is today generally believed by health workers that "the physiologic problems of ordinary ventilation have ceased to be chemical and pulmonary, and have become physical and cutaneous." The work of recent years has showed that all the effects of poor ventilation can be reproduced under experimental conditions by overheating, excessive moisture, and lack of air movement. Subjects have been placed in experimental chambers and kept there while the products of respiration accumulated. The subjects eventually manifested all the symptoms of poor ventilation. Various means were then used to relieve the symptoms. They were permitted to breathe fresh air through tubes connected with the outside while their bodies were bathed in the vitiated air of the chamber. This did not relieve the symptoms due to poor ventilation. However, subjects were completely relieved when the air of the experimental chamber was cooled even though they were breathing the same foul air which a few moments before was causing them such distress. In other tests the carbon dioxide was permitted to accumulate to such an extent that a match would not burn and the distress became very pronounced, yet the subjects were all relieved when the air was set in motion by means of a fan. In still other tests people outside were permitted to breathe from the vitiated exptrimental chambers through a mouthpiece while their bodies were bathed with the fresh cool air of the outside. They felt no discomfort. These same individuals immediately became uncomfortable on entering the experimental chamber. Hence, the conclusion has been reached that the feeling of discomfort and lassitude experienced under poor ventilation is probably due directly to anemia of the brain and other internal organs caused by the pooling of the blood in the capillaries of the surface of the body. This manifests itself by a measurable increase in body temperature and a lowered immunity which may persist for hours after the condition which

caused it is relieved. The far-reaching effect of excessive temperature is thus summarized by Winslow: "A temperature in excess of 68° F., with little air movement and moderate humidity; exerts direct and important effects upon the circulation, causing particularly an increase in body temperature, a rise in heart rate, and a fall in vasomotor efficiency; it profoundly limits efficiency by causing a disinclination to physical exertion; and it markedly increases susceptibility to disease." For a room to be properly ventilated the air coming into it must be free from dust and injurious fumes, kept in slight but not observable motion, have a humidity of approximately 50 per cent, and a temperature of from 62° to 68° F. The temperature must vary with the occupant; very young, old, or ill individuals will require a higher temperature than do others. Individuals engaged in physical activity require a lower temperature than those engaged in sedentary work. It is evident from this that the thermometer is essential in ventilation, and "no school room, no factory work room, no office, no living room should be considered as furnished and fit for human occupancy without a thermometer." This is stressed by a New York City regulation requiring that a large thermometer, with a heavy red line opposite 68° F., be placed in a conspicious place in every school room.

CHAPTER XXXIII

THE INTESTINAL BACTERIA

We are living in a world teeming with bacteria. They are on everything we touch. Practically every bit of food and every drop of water we consume contains few or many bacteria, depending on the past history of these foodstuffs. What is the fate of the microbes after they enter the alimentary canal? Are they killed? Do they multiply or remain dormant? May they be reduced in numbers or replaced in kind? Do racial dietary habits influence the microbial germs? Do they help or do they hinder? Is man what his microbes make of him? Alcohol is a microbial product which, if given to man in small quantities is a stimulant, in larger quantities a depressant, and in still larger quantities it may be a fatal poison. This is true of many poisons, including those produced by pathogens. Do the saprophytic and parasitic microbes which swarm in the alimentary tract of man produce stimulants, depressants, or highly potent poisons? Each specific plant produces its peculiar fruit; one does not expect to "gather grapes of thorns nor figs of thistles," consequently, the products vary with the microflora.

Number.—The mouth is a microbial garden, the number and variety of microbes varying with each individual. The well-kept mouth, free from disease and containing a set of healthy teeth may contain few; whereas the mouth filled with decaying teeth, each cavity and crevice filled with decaying and putrefying food, contains many. The bacteria of the mouth, together with those in the food and water, are carried into the stomach. Here they meet the acids of the gastric juice which stop all multiplication and destroy most of the least resistant micro-organisms. Hardy spores, micro-organisms imbedded in masses of food, and those which reach and leave the stomach when acid secretions are in abeyance, or when the acid is neutralized by proteins find their way into the intestines. Here conditions of food, warmth, moisture, and reaction are ideal for microbial growth. Relatively few bacteria pass the stomach, and the sojourn in the duodenum is comparatively short; hence, the microflora of this part of the intestines is sparse. Cushing

and Livingston have called attention to the phenomenon that gunshot wounds in this region are far less likely to develop peritonitis than are wounds lower down, for few bacteria enter the abdominal cavity from duodenal wounds. This, in a measure, accounts for the difference. From the duodenum to the ileocecal valve the number of bacteria increases rapidly, but the maximum is probably not reached until the ascending colon. Beyond this, the number of living bacteria rapidly decreases, for the feces contain approximately 90 per cent dead bacteria. The numbers excreted daily by a healthy adult on a mixed diet have been variously estimated from one hundred to thirty-three hundred billions. This number of dry bacterial bodies would weigh more than 5 Gm. and would contain about 0.6 Gm. of nitrogen. This is approximately 42 per cent of the total fecal nitrogen.

Kind of Bacteria.—It is evident that the enormous number of bacteria excreted daily cannot represent those taken in with the food which really contains relatively few, but rather indicates that rapid bacterial multiplication occurs in the alimentary canal. The kind of micro-organisms and rapidity of multiplication vary in different individuals, depending largely upon the nature of the food.

The alimentary canal of the child at birth is sterile. During the first few days, which Kendall refers to as the period of "adventitious bacterial infection," the child obtains various micro-organisms from the air and the things with which he comes in contact. These bacteria, however, are only temporary inhabitants of the intestines and are soon replaced by others. The kind that eventually becomes established depends very largely on the food of the child.

The upper part of the small intestines of breast-fed children contains *Streptococcus faecalis*, *S. lactis*, and a general predominance of the coccoid form. Lower down and before the ileocecal valves, the *Aerobacter aerogenes* and the *Escherichia coli* appear. In the lower part of the colon and the rectum, *Bacillus bifidus* and similar anaerobes predominate. Many proteolytic bacteria also may be present. In the upper bowels of the artificially fed child occur members of the colon and aerogenes groups, and considerable numbers of *Bacillus mesentericus* and other anaerobic spore producers; but in the lower bowels are large numbers of the *Lactobacillus acidophilus* and related organisms.

As adult life is attained greater numbers of *E. coli* and *B.*

mesentericus occur along with several species of gas producers. Often *E. coli* constitutes 75 per cent of the bacterial flora. The alimentary canal of an individual on a mixed diet, especially if it is rich in protein, contains putrefiers *Proteus vulgaris, Clostridium welchii,* and *Cl. putrificum.* These organisms are supposed to be associated with the production of certain toxic substances which give rise to so-called "auto-intoxication." However, this has not yet been proved. In the presence of excessive quantities of carbohydrates a diarrheal condition may be caused in children by the irritating products produced by *Cl. welchii.* According to Jordan many instances of anemia in children and adults seem to be accompanied by a chronic infection of the intestinal tract with this organism, and as the general condition of the patient improves there is a distinct reduction in the numbers of this organism that can be found in the feces.

The relationship of the host to its intestinal microflora is summarized by Kendall as follows: "The prominent types of bacteria that appear in the intestinal flora of a normal person are rarely constant in their occurrence, but there may be a well-marked seasonal and even annual variation in the relative proportion of the individual groups of organisms which comprise this flora. This suggested that the normal bacterial flora is acclimatized to the intestinal environmental conditions of temperature, reaction and composition of food, and of intestinal secretion at different levels. It also indicates that the activities of the organisms which comprise the normal intestinal flora are not in active opposition to those of the host."

Function of Intestinal Bacteria.—The question has often been raised: Are the intestinal micro-organisms essential for the satisfactory digestion of food, or should they be regarded as true parasites? Pasteur believed that they are essential. "I do not conceal the fact that had I time to undertake this study I should do so with the preconceived opinion that life under such conditions would become impossible (*i. e.*, without intestinal bacteria). Given that such experiments are capable of being gradually simplified, it might be possible to study digestion by adding systematically to the sterile food of which I speak various individual bacteria or different bacteria together, each of definite known species.

"The hen's egg lends itself without serious difficulties to experiments of this kind. Previously freed externally from every sort of living impurity just before hatching the chick should be immediately put into a chamber free from every sort of bac-

terium so that it could be supplied with pure air and sterile food easily introduced from without in the shape of water, milk, and corn.

"Whether the results were positive, confirming the preconceived idea which I am putting forward, or negative, or even absolutely opposite, *i. e.*, life without bacteria is easier and more vigorous, in any case the experiment would be full of interest."

Several investigators have attempted to conduct experiments in the manner suggested by Pasteur. The conclusions are uniform in one respect—that the experiments are beset with far greater difficulties than Pasteur anticipated. Nuttall and Thierfelder tried the following experiment: Guinea-pigs were removed shortly before birth from their mothers by cesarean sections. Suitable precautions were taken so that they did not become infected. They were then placed into sterilized cages, given sterile food, water, and air. Such animals gained in weight normally. Later, experiments of the type Pasteur had suggested were conducted on chickens. The surface of eggs was carefully freed of bacteria, incubated and hatched in germ-free incubators. The resulting chicks were kept in a germ-free environment, given sterile food, water, and air. These chicks ate well but did not make proper growth. Similar experiments were conducted on turtles and on frogs. The results in many cases were inconclusive. Probably in a large measure the inconclusive results may have come from the feeding of inadequate diets. As it is conceivable of a diet being sufficiently balanced when fed to animals with an intestinal microflora and insufficient when fed to animals with a sterile alimentary canal, the results of Levin are more convincing. He found the intestines of a number of Arctic animals practically sterile, yet they functioned normally.

Loeb and Northrop's experiments on the fruit fly shed considerable light on two questions: (1) Are intestinal bacteria essential for normal life? (2) Do bacteria growing in the alimentary canal elaborate poisons which injure the body of the host? Freshly laid fly eggs were placed for a few minutes into a bichloride of mercury solution of sufficient strength to kill all the micro-organisms on the surface. Only a few of the eggs thus treated survived. These were kept in sterile flasks on sterile meat and developed into normal flies. These were carried through 87 aseptic successive generations on sterile yeast. At the close of the experiments their bodies were analyzed and found to be sterile inside and out. They were apparently normal in

every respect except that they were free from micro-organisms. Their average span of life was twenty-eight and five-tenth days. Pearl found the average life span of nonsterile flies grown at the same temperature to be thirty-one days. Similar results have been obtained by others. Consequently, the conclusion has been reached that natural death is not, as suggested by Metchnikoff, due to poisons elaborated in the intestines by bacteria, but rather that old age and natural death are caused either by the gradual production in the body of sufficient quantities of harmful or toxic substances, or by the gradual destruction of substances required by the body to keep it in youthful vigor. Under natural conditions both factors may contribute their part in causing old age and death. Consequently, it may be concluded that intestinal bacteria are not essential to digestion and normal health. Still, it is evident that they do play a beneficial, if not vital, rôle in life. Naturally some harmless and some injurious bacteria will reach the alimentary canal. Now, it is well-known that the products of some bacteria, particularly those which produce acids, will inhibit the growth of others; consequently, intestines seeded with harmless parasites are protected against injurious ones just as a well-seeded meadow is protected against weeds.

Injurious Action.—It is quite a general experience that constipation gives rise to certain symptoms which consist in the main of mental haziness, "dopiness," malaise, headache, coated tongue, poor appetite, and so-called "biliousness." These have been ascribed by different individuals to various causes. Johann Kampf taught that all diseases are due to fecal impaction, and this idea was held by many writers of the eighteenth and nineteenth centuries. Its greatest champion was the Russian bacteriologist, Metchnikoff. He and his followers maintained that many bacteria grow in the intestines and elaborate certain toxic compounds: Hydrogen sulphide, indole, skatol, phenols, cresols, histamines, and others which are absorbed from the bowels, circulated in the blood, and cause the symptoms. Moreover, it was claimed that the constant entrance of these products into the blood gave rise to nervousness, hardening of the arteries, and changes in the kidneys and other vital organs. Metchnikoff and co-workers looked upon the large intestine as a useless and injurious organ which had developed in the evolution of man. Lane attempted to remedy this defect by spectacular surgical procedure, but this was accompanied by such a tremendous mortality that at the present time it is resorted to only under neces-

sity, *i. e.*, in diseased conditions of the colon. Metchnikoff's observations led him to advocate a different procedure. He knew that lactic acid has a preserving effect, and as we have learned, is often used even today in the preservation of certain foods. Furthermore, it was known that the Bulgarians appear to enjoy a somewhat unusually long span of life. They consume large quantities of sour milk; hence, the conclusion was that drinking sour milk prevents putrefactive changes in the intestines which if permitted to occur would result in illness and premature death. Thus, it became quite a common practice to prescribe sour milk with the belief that by so doing the natural intestinal microflora was being supplanted by that of the *Lactobacillus bulgaricus*. Later bacteriological work has shown that although this organism grows readily in milk, it does not become established in the intestines. This has led to the use of other bacteria especially *Lactobacillus acidophilus*.

Alvarez maintains that the symptoms associated with constipation and referred to as auto-intoxication may in the child be caused by the great absorption of toxic products from the intestines, but this cannot explain the whole condition in the adult for the following reasons: (1) A great amount of work has been done in an attempt to isolate substances which are produced by intestinal bacteria and which will produce the symptoms associated with auto-intoxication. Investigators have reported the finding of such products, but others have failed to confirm these findings. Hence, it would be wrong to state that bacteria produce no such toxic products in the intestines, but it can be stated that the search has failed to reveal such a product. (2) Increased blood pressure, which is believed by some to be caused by auto-intoxication, is met with more commonly among boys than among girls, yet constipation is more prevalent among the latter. (3) In constipation the fecal mass is dried out; hence, bacterial activity and absorption of toxic products would be less than in diarrhea. (4) The lower part of the bowels absorbs from the blood and does not excrete into the blood, consequently, bacterial products to be injurious must be produced and absorbed before the material reaches the colon. (5) Symptoms similar to those associated with constipation have been produced by packing the lower bowel with inert substances. These cannot be absorbed but give rise to an antiperistaltic action with the resulting symptoms of constipation. (6) Relief comes almost instantly on emptying the bowels, a condition which could not be expected if poisonous products had been

absorbed, for it would then require time for their removal from the body. Even Alvarez agrees that it is very probable that at times there may take place a production and an absorption of harmful toxic products from the digestive tracts, but this is much rarer than the literature on the subject would lead one to believe. As in the past, the term "auto-intoxication" has been used to include all poorly understood diseases for which there is little or no evidence of their being due to poisons produced in the intestines.

The diet has a marked effect upon the intestinal microflora and regulates an individual's sense of well-being. This is vividly shown by the experiments on monkeys by Herter and Kendall. It is known that eggs and meat encourage the growth of putrefactive bacteria in the colon, whereas milk and glucose stimulate the growth of fermentative lactic acid forming organisms. Monkeys were placed first on a diet which would favor putrefaction, and then later changed to one which would favor fermentative changes. When placed on the meat and egg diet the animals became sleepy and rested their heads upon their hands in a bowed position. They were stupid and responded slowly to external stimuli. They took their food very deliberately and manifested little interest in their surroundings. Not infrequently after a hearty meal they would spend much time in trying to bite the woodwork of the cage. The urine was of small volume and amounted approximately to one half that produced on a milk-carbohydrate diet. The amount of putrefactive products in the urine was increased. When the animals were changed to the milk-glucose diet, both the physical and psychical attitudes underwent a great change. They no longer held their heads in their hands but assumed an upright position. Their appetites were keen, their eyes became bright, they showed more interest in their surroundings, and the putrefactive products in the urine decreased.

Influence of Putrefaction on Infection.—Metchnikoff believed that some micro-organisms may assist others in entering the body from the intestines. He made streak inoculations on gelatin plates with cholera organisms. Crosswise of these he inoculated certain putrefiers. At the points where the lines crossed larger colonies of the cholera germ were formed than elsewhere on the plates. Repeating this experiment with acid producers there was no growth of the cholera organisms at the points where the lines crossed. Consequently, he concluded that putrefiers accelerate the growth of cholera germs, whereas acid pro-

ducers inhibit. He next conducted experiments on animals. Guinea-pigs, rabbits, cats, and other animals are refractive to cholera, but Metchnikoff found on feeding young rabbits putrefiers and then the cholera germ, that these animals developed a disease similar to cholera. Furthermore, Meyer has found that guinea-pigs, which are normally immune to the typhoid bacilli that enter through their mouths, become infected if they receive the germs when they are on a diet of bread and milk.

It is general information that the intestines of the child are more permeable to bacteria than are those of the adult, and it is maintained by Park that much of the injury resulting from the feeding of poor grades of milk during the summer months comes from certain bacteria which are normally nonpathogenic permeating the intestines. This may result from lowered vitality brought about by heat, associated with micro-organisms, or possibly there may be certain vitamin deficiencies. Nevertheless, it opens up a new and interesting lead as to the cause of certain heretofore ill-understood disorders which in the past have been attributed to auto-intoxication. Hence, Adami and others feel that it is much more rational to regard the ill effects of intestinal stasis in man as caused not by auto-intoxication, but by conditions favoring subinfection.

How to Modify Intestinal Microflora.—Numerous attempts have been made to decrease or to change the intestinal microflora. Sterile food has been fed and found to have little effect in reducing the number of micro-organisms of the intestines. Complete starvation may reduce the number of bacteria in the upper level of the intestines but there are reported cases in which there is an actual increase. Many intestinal antiseptics have been tried (and some are still being used), but practically all of those workers who have taken the trouble to make bacterial counts before and after the administration of these drugs admit that they have little or no effect.

Numerous investigators have attempted to control intestinal putrefaction by feeding acid-forming bacteria together with carbohydrates. Escherich used *Aerobacter aerogenes.* Quicke suggested the use of yeast, *Oidium lactis,* and the writings of Metchnikoff gave this method a great impetus. He considered the failures of others are due to the use of organisms having weak acid-producing powers; consequently, he advocated the use of *L. bulgaricus* which was isolated from milk and has been proved to produce as much as 25 Gm. of lactic acid per liter of milk. He advocated the use of the organism either in milk or

broth cultures. His enthusiasm, together with the simplicity of the process, has almost made a fad of it in some localities and with some persons it is considered a cure-all. Yet the mere feeding of *L. bulgaricus* for the purpose of antagonizing putrefactive processes in the intestines has been disappointing. It has been found that although the organism grows readily and produces large quantities of acid in the test tube, it fails to become established in the intestine.

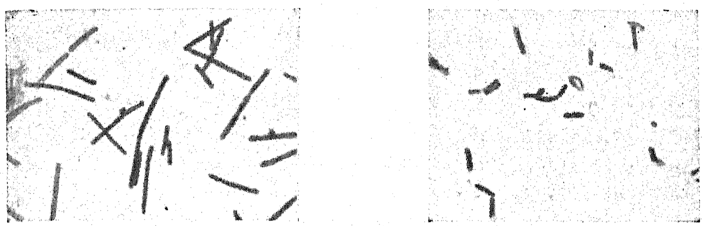
The use of *L. acidophilus,* which is a normal inhabitant of the digestive tract, seems to be much more promising. Extensive work has shown that the intestinal microflora can be changed not only by giving massive cultures of *L. acidophilus* but also by giving large quantities of lactose. The lactose is so poorly absorbed from the bowels that it gets down into the lower ileum and colon and there modifies the culture medium to such an extent that the sugar-fermenting bacteria can outgrow the putrefactive ones.

Kopeloff has been able to change quite permanently the microflora of the intestine by feeding milk containing massive doses of *L. acidophilus.* He used 1000 cc. of a culture containing 200,000,000 viable *L. acidophilus* organisms per cubic centimeter. By this means he was able to overcome bad cases of constipation and to prove that the effect was bacteriological and not physical, or chemical. He showed: (1) That patients given large quantities of sterile milk were not relieved of constipation; (2) that the feeding of large quantities of milk containing the dead bodies of *L. acidophilus* and their products failed to relieve constipation; and (3) that *L. acidophilus* milk from which the organisms had been separated by filtration also failed to cure constipation. But, when milk was fed containing massive doses of the living *L. acidophilus* organism, the results were always favorable. Most authorities are agreed that the tablets and other preparations on the market are practically valueless, as may be seen from the following statement of Bass:

"In the instance of tablets, none of these examined was found to contain as much as 1000 viable bacteria of any kind per tablet. If it should be granted that all the viable bacteria present were *L. acidophilus,* it would take about 1,000,000,000 tablets, or more than 20 tons, to contain as many bacilli as are contained in 1000 cc., or the usual daily dose of the acidophilus milk, the quantity found, by most investigators at least, to be necessary to transform the flora."

Similar results were obtained in liquid cultures which are often

attractively labeled and tastefully flavored but contain so few bacteria that a patient would have to drink about 7 to 8 gallons daily to get the required dose. Consequently, where the organisms are to be used, the starters must be obtained from reliable laboratories and grown in milk under appropriate conditions. Encouraging as have been the results in modifying the

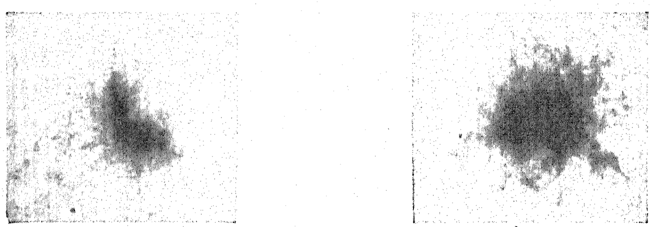


a b

Fig. 106.—(*a*) *L. bulgaricus*, (*b*) *L. acidophilus*. (*After Kulp and Rettger.*)

intestinal microflora by *L. acidophilus*, the most effective and most permanent method is still the use of a suitable diet, particularly one containing large quantities of lactose and dextrin.

As regards the *L. acidophilus* it might be well to add that it is a gram-positive bacillus which easily undergoes granular degeneration. It grows under acid conditions not tolerated by most



a b

Fig. 107.—(*a*) Agar colonies of *L. bulgaricus*, (*b*) agar colonies of *L. acidophilus*. (*After Kulp and Rettger.*)

other bacteria. It is a nonmotile rod, 1.5 to 2 microns long and 0.6 to 0.9 micron wide with slightly tapering ends. The optimum temperature for growth is 37° C. In milk, growth occurs slowly, taking about three weeks for complete curdling. It produces no gas and does not form spores. It is nonpathogenic for animals.

Dental Caries.—Dental caries is as old at least as civilization. Ancient Egyptian mummies show unmistakable evidence of its existence. Both ancient and modern writings refer to it, and numerous theories have been evolved to account for dental caries; but the one of greatest interest from a bacteriological standpoint is that championed by Willoughly D. Miller. Miller believed that many bacteria produce lactic acid in the decomposing of certain carbohydrates in the mouth. Starch was considered to be the main offender because being insoluble it lodged between the teeth and thus offered a local foothold for the production of lactic acid. This he considered dissolved the inorganic salts. The organic cementing material he believed was dissolved by enzymes secreted by micro-organisms. The result of this dual activity is a cavity in the tooth. Later work by Bunting and co-workers points to the conclusion that members of the acidophilus group are of importance in the decalcification of dental caries. The organisms encountered are apparently different from acidophilus varieties found in the intestines, and the term *Lactobacillus odontolyticus* has been applied to the variety found in dental caries. They have further demonstrated that certain antiseptics, such as metaphen and hexylresorcinol change the bacterial flora of the mouth so that the acidophilus organisms almost disappear. This, however, is only one factor in dental caries, two others being defects in the histological structure of the teeth and an inadequate diet.

CHAPTER XXXIV

BACTERIA AS THE CAUSE OF DISEASE

BACTERIA govern the length of man's life and determine the part of the earth on which he may live. They have defeated armies and destroyed cities. Nations which have been the glory of the world have gone down before them. The majority of individuals born today would enjoy health and live to old age were their bodies not invaded by microbes. They are no respecter of persons, nor are they governed by the edicts of rulers. However, at the command of modern science they are commencing to relinquish their hold, and some of earth's most fertile regions, where in the past the risk of death to the visitor was greater than a visit to a battlefield, are being transformed into tropical health resorts. Some microbes are no longer the fairy tale adventures of death and disease. They are being controlled; disease is being reduced; and during the past century man's expectancy of life has been doubled. Still much remains to be done, for the learning and practicing of the laws of sanitation can prevent more suffering and save more lives than the abolition of war. Here the scriptural entreaty is especially appropriate: "Give me understanding and I shall keep the law; yea, I shall observe it with my whole heart." Each bit of knowledge gained in this field carries man one step nearer the goal where he will be the master and not the slave of his environment.

The Germ Theory.—Primitive man had no conception of infectivity as we understand it today. He pictured demons and other material entities as the cause of disease and hence attempted to prevent and cure them with charms and incantations. Yet throughout the ages there has appeared, like a silver thread interwoven with this warp and woof of mystery and skepticism, the occasional unheaded word of the genius proclaiming them communicable.

Casual allusions to contagion are to be found in the writings of Lucretius, Vergil, Livy, and Plutarch. Isocrates states that certain diseases are communicable, and Varro in the first century B. C. suggests that malaria may be due to minute invisible organisms. Ovid wrote: "Bodies rot on the ground, blasting

with their stench and spreading contagion far and near. No one can control the pest, which fiercely breaks out upon the very physicians, and their arts do but injure those who use them. The nearer one is to the sick and more faithfully he serves them, the more quickly is he himself stricken unto death."

In ancient Egypt there were vague ideas of an association between rats and pestilence, and it was customary to encourage snakes in houses with the object of keeping them away. In the Pentateuch we find the injunction: "All the time that the plague is on him he shall be unclean. He is unclean, he shall dwell apart, without the camp shall his habitation be." Disinfection is enjoined, and various methods, such as washing, burning, the use of animal charcoal and the scraping of the walls are given.

During the Middle Ages the idea of infectivity of some diseases became even more prevalent. Experience was teaching that not only leprosy but also plague, consumption, itch, erysipelas, conjunctivitis, and fevers associated with rashes were contagious. Venice, a city whose commercial supremacy was almost entirely dependent on its sea-borne traffic, used an adjacent island on which to detain persons coming from places where the plague was prevalent. They were detained at first for thirty days; later it became the custom to detain them for forty days; hence, the origin of our term "quarantine."

In the premicroscopic days of the sixteenth century an Italian poet and physician of Verona, Fracastoro, propounded a rational theory of infection which sounds modern. He spoke of invisible seeds of contagion capable of multiplication and of being passed from the ill to the well. He recognized the contagiousness of tuberculosis and made an analogy between infection and fermentation.

Nearly one hundred years after the discovery of bacteria by Leeuwenhoek, Marcus Antonius von Plency propounded a germ theory of disease. He believed that all infectious diseases were caused by micro-organisms which were living and self-propagating. On these bases he endeavored to explain the variation in the incubation period of the different infectious diseases. He maintained that there were various germs, to which were due the specificity of the different diseases.

Henle, in his essay on "Miasma und Contagia" published in 1840, maintained that diseases are due to germs. He had searched in typhoid cadavers, smallpox material, and scarlet fever scales for these theoretical causes of disease without success. He relinquished the quest but never the idea that diseases

are due to germs. He believed his failure was due not so much to the minuteness of the germs as to their similarity to the tissues in which they were embedded. A generation later when Henle's pupil, Robert Koch, introduced fixing and staining methods and bacteria were shown to be the cause of disease, the almost forgotten Henle was proclaimed a prophet.

Up to this time, and indeed until the time of Pasteur, opinions as to why diseases spread had swayed between two theories: (1) Diseases arose from some common climatic terrestrial condition or defect of bodily constitution, or (2) diseases were spread from one case to the other by direct contact. The health workers of the eighteenth century, without completely abandoning the first, stressed the second and concentrated their efforts against the two main channels by which they believed diseases were conveyed: (1) The "effluvia" or odors from the sick persons, and (2) the "fomites," minute infective particles which were found in houses and clothing. The first could be dealt with by the free circulation of fresh air, the second by soap and water, heat, vinegar, or sulphur. Hence, Mead's pithy aphorism: "As nastiness is a great source of infection, so cleanliness is the greatest preventive."

The isolation of specific germs, their growth in laboratory media, the artificial production of disease, and the discovery of curative and preventive antitoxins and vaccines during the last part of the nineteenth century, transformed the ancient mystical theory of demonology into the modern science of bacteriology.

Koch's Postulates.—Jacob Henle in 1840 stated quite clearly the germ theory of disease and formulated the dictum: "Before microscopic forms can be regarded as the cause of contagion in man they must be found in the contagious material, they must be isolated from it and their strength tested." A generation later his illustrious student Robert Koch, in one of the most important papers he ever wrote, presented his views as to the condition which must be met before it can be stated that a specific micro-organism is the cause of a disease. As translated by Lutman, he wrote:

"My viewpoint may be briefly characterized as follows: It has not yet been shown that all infectious diseases are conditioned by parasitic micro-organisms, so, in every instance, proof of the parasitic character of the disease must be offered. The first step in this proof is a careful investigation of the parts of the body changed by the disease in order to establish the presence of the parasites, their distribution in the diseased organs, and their relation to the tissues of the body. It is understood that for this investigation, every means which the new microtechnic offers

is to be brought into service. The tissues and their humors, blood, lymph, etc., in a fresh condition, as well as without and with reagents, are to be investigated microscopically. They are to be dried on the cover glass and stained by various methods. The imbedded objects are to be sectioned on the microtome, stained, and the slides subjected to careful scrutiny with the use of the best adapted lighting methods and lenses. After this fundamental orienting, it is possible to decide whether microorganisms occur in the diseased parts and as to the parts where they appear in pure culture; whether, for example, they are to be found in the lungs, spleen, blood of the heart, etc. After their presence has been established and their pathogenic nature, that they are the cause of the disease which has arisen can then be decided. For this purpose, they are to be grown in pure cultures and freed from all adhering portions of the diseased body. They are to be inoculated back, if possible, into the same species of animal in which the disease was observed, or into such animals as it might be expected to occur with recognizable symptoms. In order to make this clear, I will cite tuberculosis. First, through a microscopic investigation, I established that the diseased organs were filled with bacilli of a special staining reaction; these bacilli were then isolated into pure cultures out of places where they were not mixed with other species, and were purified in this procdure. Finally, it was demonstrated that by the inoculating back of such pure cultures into numerous animals of various species whose susceptibility to the disease was known, tuberculosis could be produced anew. A second very instructive example is offered by erysipelas in man. It has been known for a long time that in this disease the lymph glands of the skin always contain certain micrococci. But it was not yet proved that they were the cause of the disease. After Fehleisen had succeeded, however, in growing these micrococci in pure cultures, excluding all chance organisms which occur on the surface of the skin, and by inoculation had succeeded in producing in man typical erysipelas, there can be no doubt that these micrococci were the cause of erysipelas; and that the latter is to be regarded as a parasitic disease." *

The essence of this paper by Koch has been incorporated into the canons of bacteriology, known as Koch's Postulates or Laws. They are variously stated. Topley gives them as follows:

"1. The organism should be found in all cases of the disease in question, and its distribution in the body should be in accordance with the lesions observed.

"2. The organism should be cultured outside the body of the host in pure culture, for several generations.

"3. The organism so isolated should reproduce the disease in other susceptible animals."

Erroneous conclusions have been reached when only parts of the laws had been applied. For example, *Salmonella suipestifer* at one time was considered the cause of swine fever; today we know the cause is a filterable virus. In still other cases, secondary invaders may enter and be found so general and corres-

* From B. F. Lutman: Microbiology, McGraw-Hill Book Co., Inc., Publishers.

ponding to the lesions so closely as to lead to the belief that they are the specific cause. This can be settled, where possible, by an application of the third postulate. In the early days of bacteriology the proposition that the causative organism of a disease should be present in every case adequately examined was held to imply the converse—that the organism should be found only in the person suffering with the disease. Loeffler not only found *C. diphtheriae* in the throats of individuals suffering with diphtheria but also in the throat of an apparently healthy child. This discovery made it doubtful if the causative organism had been discovered. Topley has suggested that the first postulate must imply: "Our present conception of the probable distribution of any pathogenic parasite is that it will be found in every case of the specific infection that is clinically obvious, and is adequately investigated under satisfactory conditions; in many cases of minor infection that show little resemblance to the fully developed disease; and in a certain proportion of apparently healthy persons, especially in contacts."

It may be proved by animal inoculation that an organism is associated with a disease and that its distribution conforms to the lesions, yet it may not be possible to observe the organism with the microscope or even the ultramicroscope. In these cases it is impossible to meet the second postulate by growing the virus in pure cultures. Some diseases are peculiar to man, or if artificially produced in lower animals give a picture far different from that occurring in man. Occasionally in such cases laboratory accidents have supplied the needed information. This has occurred in typhoid and undulant fever. In still other cases we must be content with the demonstration of specific antibodies in the blood of infected man or animal. Hence, there are numerous communicable diseases known in which Koch's postulates have not been fully met, yet we feel reasonably certain of the specific causative micro-organism.

Pathogenicity.—Continually surrounded as we are by bacteria, the mystery which confronts the beginner in bacteriology is not why men die of microbial infection, but really how is it possible for any of them to escape death for so long a period. The student soon learns that this is due to a number of reasons and that bacteria can be divided roughly into three classes: Saprophytes, parasites, and pathogens. While the last two classes overlap, yet there is a marked difference in the invasive powers of various parasites. With few exceptions, all pathogens are parasites, yet all parasites are not necessarily pathogens. Nevertheless, the classification has considerable merit.

Saprophytes live on dead organic matter and transform it into such forms that the higher plants and animals can use it again. Saprophytes are the most numerous and most important forms of microscopical life. These bacteria comprise that great group of micro-organisms (chief among which are fermenting, putrefying, and decaying bacteria) that play such a prominent rôle in the cycles of the elements. They are widely distributed in nature and many of them form resistant spores which carry them over long adverse periods, they rarely initiate a disease in man or the lower animals but are occasionally found associated with diseased conditions as secondary invaders. They usually gain their foothold and do their damage after other micro-organisms or conditions have broken down the natural barriers. Occasionally, as in the case of the diphtheria and tetanus bacilli, they may grow and produce toxins on a localized area of dead tissue and in this manner produce disease; or, as in the case of *Clostridium botulinum,* they may elaborate a toxin in foodstuffs outside the body, disease resulting when it is eaten with the food.

A smaller group is that of parasitic micro-organisms which exist upon the body of living plants and animals. They invade and give rise to disease when the natural barriers are broken down. They are seldom found far from the bodies of their hosts. The colon bacillus is an example of this group. Its natural habitat is the gastro-intestinal tract of man and many animals. Under appropriate conditions it may invade the body of its host and give rise to appendicitis, cystitis, peritonitis, and other inflammatory processes. These parasitic micro-organisms may add the finishing touch in many diseases. To this same group belong the streptococcus, the "blood poison organism"; the staphylococcus which causes boils and carbuncles; and the various bacteria which exist in the upper part of the respiratory tract and which under favorable conditions give rise to pneumonia. This group of parasites has appropriately been designated by Theobald Smith as "opportunists." They seldom initiate a disease but are often present and enter the body as secondary or terminal invaders. They are comparatively frail.

A still smaller group is that of the parasitic pathogenic micro-organisms which attack man and the lower animals. Some of these are pathogenic only for man, others only for the lower animals. A few cause disease in both man and the lower animals. With few exceptions, they can multiply only in the body of their host and pass rather quickly from the body of one animal to that of another. Probably they have evolved from the

other parasites, which in turn had their origin as saprophytes. The sequence of events probably was: Saprophytes, parasites, parasitic pathogens. It is quite probable that the saprophytic bacteria were the first to render the surface of the earth fit for higher plants, which in turn furnished food for animals. These same saprophytes may then have accidentally reached the surface of the bodies of living plants and animals, where most of them were dislodged or died, while a few adapted themselves to their new environment and became parasites. Eventually some of the latter may have invaded the living tissue and have become pathogens. Such a theory raises the probability of new parasites and new pathogens being evolved even at the present time.

The nature and extent of the disease produced by a pathogen, according to Kendall, depends upon the following factors: (1) The kind of micro-organism; (2) the number of micro-organisms; (3) their ability to locate and force an entrance into the tissues of the body; (4) the location and the extent of their multiplication in the tissues of the host; (5) the reaction of the tissues of the host to the invader; and (6) the nature and extent of the secondary specific defenses of the host against the invader.

The disease-producing ability or virulence of micro-organisms is susceptible to variation. In many cases it may be increased or decreased. Pasteur increased the virulence of rabies virus taken from dogs for rabbits by passing it successively through the bodies of rabbits. Usually when passed through the bodies of specific animals bacteria increase in virulence for that species of animal and decrease for others. Pasteur also found that those micro-organisms which cause chicken cholera when grown on laboratory media, as well as the anthrax bacilli grown at an abnormally high temperature, decrease in virulence. Organisms which are reduced in virulence by any means are referred to as attenuated. Attenuated micro-organisms are often used in rendering animals immune to disease.

How Pathogens Reach the Body of Man.—Most pathogens multiply only in the body of man and in the lower animals; hence, they must leave the body of the diseased and reach the body of a healthy animal before they can initiate a disease. Before they enter the body of man, they must pass both mechanical and chemical barriers, which constantly stand between man and the invading hordes. Some bacteria, such as diphtheria and tetanus, grow on the body and elaborate a poison which is absorbed and which gives rise to the specific disease. Others,

like typhoid and anthrax, multiply in the body tissues. It is, thus, quite evident that the ease with which the pathogens are distributed depends largely upon the nature of the disease they produce. When, as is often the case with tubercle bacilli, they enter the bones, they cannot readily leave the body of the host and secondary cases do not occur. When pathogens, such as the typhoid bacilli, enter the blood stream and leave in the urine and alvine discharges, the likelihood of their reaching a second individual is less than when they leave in the secretion of the nose and throat, as in the case of diphtheria, scarlet fever, and influenza.

Having left the body of their first host, pathogens must find a second host or perish. While most of them perish, a few reach susceptible individuals to whom they are conveyed by direct contact—the fingers, objects which have been mouthed by the diseased, various insects, and food. Inanimate objects which are spoken of as fomites may convey the more resistant, but it is becoming more generally believed that this method of conveyance is of secondary importance, as compared with contact infection where the freshly infected secretions of the diseased are rather quickly conveyed to a second individual. It was shown in an earlier chapter that droplet infection may be conveyed for a few feet, but true air-borne infection plays little, if any, part in the spread of disease. The manner in which the organisms causing the communicable diseases are transferred from individual to individual is spoken of as the route of infection. The portals by which micro-organisms enter the body are referred to as the channels of infection and may be grouped under three headings: (1) The skin, (2) the respiratory tract, and (3) the digestive tract. Some organisms may enter by two or even three channels, but many usually enter by a specific channel.

Even when a virulent pathogenic micro-organism reaches the body in adequate numbers it may not infect, except when it enters by a specific channel. Park illustrates this as follows: If very virulent streptococci, typhoid bacilli, and diphtheria bacilli are rubbed into an abrasion of the skin, the typhoid bacilli produce no lesions, the diphtheria only a very slight area, while the streptococci may give rise to a severe or even a fatal septicemia. If the same organisms are placed in an abrasion of the throat, the typhoid is again harmless. The diphtheria organism may give rise to diphtheria and the streptococci to tonsillitis, or even to septicemia. Finally, if the three organisms reach

the intestines, it is the typhoid which causes a diseased condition, and as a rule not the others. If the tetanus organism reaches an appropriate wound on a man or horse it gives rise to the highly fatal affliction, lockjaw. Yet the normal habitat of this pathogen is the intestinal tract of the horse and often the alimentary canal of man.

According to Rosenau, approximately 90 per cent of all infections enter the body through the mouth with the various objects which are placed in it—fingers, eating and drinking utensils, food, water, and so on. This is probably one reason why children have more communicable diseases than adults. It also makes this the most important channel to guard in preventing the spread of communicable diseases.

Mechanical Barriers.—After micro-organisms reach the surface of the body they must pass certain mechanical barriers before they enter the body and cause pathologic conditions. The outer defenses are the skin and mucous membrane. The skin is constantly covered with various micro-organisms, most of which are transient saprophytes that come and go irregularly. A few, such as the pyogenic cocci, occur with such regularity as to be considered provisionally as habitual parasites. To these hordes which hover about us, the skin presents an almost impenetrable barrier. Some cocci and plague bacilli, when vigorously rubbed into the sweat glands and hair follicles, may penetrate the underlying tissue and cause disease; but this is exceptional, even with these, for they usually enter through some cut or abrasion, a severe wound, the slight lesion of a hang nail, or the tiny bite of an insect. In addition to the pyogenic cocci, the organisms which cause tetanus, anthrax, symptomatic anthrax, malignant edema, and the organism which causes gas gangrene occasionally enters the damaged skin and cause either localized infection or widely distributed injury.

The skin functions mainly as a mechanical barrier, yet it is becoming evident that the efficiency of this barrier is enhanced greatly by a complex biological mechanism which enables the skin to rapidly free itself of foreign bacteria. Hemolytic streptococci, *Proteus vulgaris*, Friedländer's pneumobacilli, and *Escherichia coli* die much sooner on clean hands than when exposed on glass or other inanimate objects. Some investigators failed to recover *Escherichia coli, Eberthella typhosa* and *Salmonella enteritidis* from the palm of the hand ten minutes after they had been placed there. This germicidal property quickly disappears at death. Dirty hands do not possess this auto-sterilizing power,

but it quickly appears on washing. Consequently, clean hands serve as one of our greatest protections against infection, since the washing with soap and water not only tends to remove occasional pathogens but also increases the skin's germicidal properties. This autosterilizing action is not effective against staphylococci and other normal bacterial inhabitants of the skin. Furthermore, Colebrook found that no amount of washing, nor any of the chemical disinfectants commonly employed for sterilizing the hands in obstetrical practice, removes from the skin the numerous staphylococci and less numerous diphtheroid bacilli that normally vegetate thereon.

Because of their moisture and warmth, the mucous membranes covering the inner surfaces of the body occasionally invite bacterial multiplication. In the case of the nose and upper respiratory passages, the moist membranes hold the bacteria, thus preventing their entering into the deeper alveoli of the lungs. Even here the healthy mucosa is a nearly perfect barrier, for most individuals harbor pathogens in the upper respiratory passages, which, if permitted to enter the tissue, may give rise to disease. The lung is often damaged by irritating fumes, sharp bits of straw, rock, and emery or glass, and pneumonia quickly follows. During health the constant rhythmic contraction of the cilia carry upward and outward the bacteria which may have penetrated the bronchi and bronchioles.

The eyes are presided over by the faithful eyelids and eyelashes which keep out many would-be intruders. The polished surface and the constant flow of tears carry some invaders out and under ordinary conditions prevent infection. When the tear ducts fail to function normally as, for example, when the individual does not receive sufficient vitamin A in his diet, lesions occur and infection becomes more prevalent. Normally some bacteria continually find their way to the eye, and *Corynebacterium xerosis* occurs with such regularity in the conjunctival sac as to be regarded as a normal inhabitant. Gonorrheal infection often enters by this channel, and in the case of the child is probably the greatest single cause of blindness. It has been shown that plague, glanders, and hydrophobia may be transmitted by installation of infectious material into the conjunctival sac. It has been claimed that there occurs in the tears, nasal secretion, and in other body secretions a *lysozyme* which lyses bacteria, but it is now quite evident that they play little if any part as a germicide.

The mouth is a veritable garden of micro-organisms, sapro-

phytes, parasites, and often pathogens; some of the latter occasionally invade the salivary glands. These organisms find a suitable home in every crevice and region of the mouth. They cause dental caries and often penetrate to the very root of the teeth and cause not only loss of teeth but often constitutional diseases. The normal secretion of the saliva washes many from the surface of the teeth and keeps them on the move. When for any reason normal secretion of the saliva fails, the teeth and gums quickly become coated with bacterial films which give rise to ill-smelling and tasting products. Moreover, individuals who constantly sleep on one side usually lose the teeth on the opposite side of the mouth earlier than those on the side on which they sleep. This is probably due more to the failure of being constantly washed by the saliva than to its slight bactericidal properties.

The microflora of the mouth is constantly being washed into the stomach where they come in contact with the gastric juice, which is highly unfavorable to their life and multiplication. This is due to the hydrochloric acid which it contains. Even this partakes more of the nature of an antiseptic than a true disinfectant. It has been established by experiments that the typhoid, tuberculosis, and dysentery bacilli rather readily pass the stomach when swallowed with food. Water and many liquid foods quickly leave the stomach with a low acidity. Moreover, bacteria may be imbedded in large particles and thereby be protected from the hydrochloric acid. Nevertheless, the gastric juice plays a prominent rôle in the destruction of saprophytes and pathogens, which in its absence would survive and reach the highly favorable medium in the intestines where they would rapidly multiply. That hydrochloric acid protects against disease is certain from the work of Koch, who found it impossible to infect some animals with certain bacteria until he had neutralized the gastric hydrochloric acid. The gastric juice destroys tetanus and diphtheria toxins but is without effect upon botulinus toxin. Investigations show the stomach to be exceptionally free from microbial infection.

Most bacteria which reach the intestines find the partly digested alkaline mass highly favorable; hence, they rapidly multiply, and by the time the mass reaches the descending colon each gram contains billions of micro-organisms.

Considerable antiseptic action was formerly attributed to the bile, but that this cannot be great is indicated by the fact that typhoid bacilli have been found growing in the bile ducts and

gallbladder. It appears to favor the growth of the colon-typhoid group of bacteria. Bile increases peristalsis and serves as a slight laxative, thus giving less time and opportunity for the pathogens to enter the body. Parasitic bacteria, like the streptococci and *Escherichia Coli,* often occur in the intestines in large numbers but normally do not penetrate the intestines. However, when body resistance is decreased, these may invade and cause disease. Some authorities consider that hot weather reduces body resistance in the case of small children and that digestive disturbances in the summer months are more often due to the entrance of parasites than to true pathogens. Even pathogenic bacteria, such as the typhoid, dysentery, and tubercle bacilli, may pass the whole length of the intestines without causing inflammation. Slight lesions aid their passage into the deeper tissue.

Number as Related to Resistance.—Even when pathogens are brought into contact with the body by a path suitable to their peculiar requirements, the number introduced must be great enough to overcome the second line of defense. The minimum number which must run the gauntlet of the body defenses varies with the micro-organisms and also with the animal. The statement is often made that "ten tuberculosis bacteria are necessary to inoculate a rabbit." Ordinarily, man can handle a few tubercle bacilli without becoming infected, but large doses are quite certain to cause infection; some other micro-organisms, for example, the anthrax bacillus and the pneumococci, may infect highly susceptible animals even when few enter. Webb and co-workers found that they could regularly cause the death of a susceptible animal with one thread of anthrax bacilli when taken directly from the blood of a dead animal, but if grown a short time on agar thirty times this quantity may at times be used with impunity. Probably in most diseases a great number of the specific microbes have to enter before the normal defenses are broken down.

Bacterial Localization.—Bacteria enter the body by specific channels and attack specific tissue. Diphtheria bacilli and haemolytic streptococci both tend to lodge and multiply in the tonsils; both produce soluble toxins, which diffuse into the blood stream and injure other tissue. Pneumococci, hemolytic streptococci, influenza bacilli, and the tubercle bacilli have a predilection for the lungs, although they may spread to other tissue. The cholera spirilla and the dysentery bacilli find conditions most suited for their multiplication in the intestinal mucosa;

consequently, the specificity of a disease comes primarily from the toxic product produced by the micro-organism, while the symptoms are associated with the tissue invaded.

How Bacteria Produce Disease.—When bacteria or their products enter the body they cause various changes within it. The more important effects are: (1) *The destruction of body cells or tissue:* Staphylococci and streptococci destroy body tissue in boils and abscesses, and in septicemia they destroy the red corpuscles. Cholera and dysentery bacilli destroy patches in the intestines; tubercle bacilli destroy the tissue of the lungs and occasionally give rise to fatal hemorrhages. (2) *The formation of true toxins:* Diphtheria and tetanus bacilli multiply at the point of entry and elaborate a poison which is discharged into the system and which causes the injury. (3) *The liberation of endotoxins or "split proteins":* Cholera, typhoid, and many other micro-organisms have within their body intracellular constituents which are retained during the life of the cell; on death, however, the poison diffuses out or is produced and injures the host. (4) *Nonspecific effects:* Fermentative and putrefactive activities of many bacteria not ordinarily classified as pathogens may act as irritants to the intestines, or their products may be absorbed, either of which may give rise to pathological conditions. (5) *Production of ptomaines:* If absorbed into the body, these may possibly give rise to illness. It must be remembered that the ill effects produced by pathogens may be, and usually are, due to a combination of different effects.

CHAPTER XXXV

IMMUNITY

BACTERIA enter the body by one channel or another. They get into the lymph, or the blood stream, and finally into the tissues. At times they gain entrance directly into the tissues as a result of a wound. They have passed the first line of defense, but here they meet other defenses. These are not mere passive obstructions, as there results an actual biological struggle between the host and the invader. Usually the conflict is a quick, decisive one, and the invader is destroyed. Often the microbe establishes a foothold, multiplies, and carries on an offensive warfare, in which case the tissues of the host mobilize their defensives, the weapons used depending upon the specific invader. In most instances a real chemical warfare ensues which ends only in the death of the invader or the invaded. There can be no armistice. On being attacked, some germs become intrenched. Then follows a waiting fight; the host constantly throws up barriers, while the invader, ever ready to take advantage of any weakening in the defense, immediately begins to wage an aggressive campaign when the weakening comes. This may end in a secondary intrenchment, the complete routing of the invader, or the death of the invaded. This eternal warfare is inherent in all animals, and the science which deals with the process is known as immunology.

Immunity is Universal.—If one examines a drop of stagnant water under the microscope it is found to contain minute forms of animal life, amebae, and minute forms of plant life, bacteria. On careful examination the amebae are seen ingesting and digesting the bacteria. Now, if one kills the amebae and again examines them the living bacteria are observed decomposing the dead amebae. It is evident that there was something inherent within the living naked mass of amebic protoplasm which protected them against the bacteria. If a living plant be carefully examined microscopically its surfaces are found covered with myriads of micro-organisms, yet the living plant is uninjured by them. If this same plant is killed and left in a moist place, the same bacteria quickly tear it to pieces. On the skin, in the

upper respiratory passages, and in the alimentary canal of man and the lower animals are millions of different micro-organisms, yet during life they do not attack. Something happens—we call it death—and the micro-organisms quickly enter all the tissues. If it is a friend who is thus attacked and we are far distant, we have to hurry to obtain a last fond look before the wrecking crew tears the body to pieces.

Then too, the germs which attack man during life are often not the ones which attack the lower animals. Animals can drink with impunity water infected with typhoid and cholera, yet man, on drinking similar water, becomes infected. Fowls do not sicken with Asiatic cholera, nor men with fowl cholera. Whooping cough and diphtheria are unknown among the lower animals, and hog cholera and Texas fever are not among the human diseases. This power of resisting infection in varying degrees is universal and is known as immunity. As may be seen from the above examples, this power is not absolute and varies with different plants and animals and under different conditions, but it is possessed by all life in some degree; for the absence of immunity, like the absence of bacteria, is incompatible with life on this planet.

Natural Immunity.—If one glances over the list of diseases to which various species and races of animals are victims, it is apparent that some animals are never naturally infected with many of the micro-organisms that cause extensive and fatal ravages in others. The lower animals are immune to gonorrhea, diphtheria, typhoid, measles, scarlet fever, and other diseases; whereas man is immune to chicken cholera, canine distemper, hog cholera, and others. This is due to natural immunity, which may be roughly defined as the immunity certain individuals posess by virtue of their belonging to a given animal species. It is inherent to a greater or less extent in all members of the species. In some cases, at least, it is due to the micro-organism failing to find suitable cultural conditions in the body of the host. For example, those germs which cause disease in the warm-blooded animals usually are without effect on the cold-blooded, and vice versa. Anthrax does not attack turtles and frogs, nor avian tuberculosis man. It has, in some instances, been found possible to infect an animal with specific micro-organisms to which it is ordinarily immune by changing its body temperature. Some diseases of the herbivora do not occur spontaneously in the carnivora. Dogs, for instance, are relatively immune to anthrax and tuberculosis.

The degree of immunity varies. In some cases it is absolute. The lower animals are immune to typhoid. Man has a fair degree of immunity to the respiratory diseases, dependent upon physical conditions. Susceptibility to these communicable diseases is greatly increased by fatigue, over-exertion, worry, undue exposure to cold or heat, poorly ventilated rooms, alcohol, diseases, and insufficient and improper food. Susceptibility to most of the communicable diseases is probably independent of the individual's physical condition.

Just how adverse conditions bring about increased susceptibility to the respiratory diseases is not well understood. Fatigue and overexertion slightly change the alkaline reserve of the blood, which may decrease immunity. Cold and overheating inactivate certain defensive substances of the blood. Moreover, it has been observed that cold paralyzes, or at least decreases to a marked extent, the movement of the cilia which cover the upper respiratory passages. Consequently, microbes are permitted to penetrate deeper into the more vulnerable terminals.

Variations in susceptibility to diseases have been observed in different races of the same species. This is known as racial immunity. It is claimed that Algerian sheep may be pastured with impunity on land so thoroughly infected with anthrax bacilli as to be deadly for the common breeds of sheep. Field mice are very susceptible to glanders, whereas white mice are immune. The black rat is more resistant to anthrax than the white rat.

Racial immunity is also perceptible in man. It is generally believed that the negro, Eskimo, and American Indian are more susceptible to tuberculosis than the white man. Carroll states that whites are more susceptible to yellow fever than negroes, and that among the latter those living nearest the equator are less susceptible than are the more northern races. Smallpox, which is so highly fatal among some races, is considered a relatively mild disease in Mexico. There seems to be no doubt in some cases that there is an actual racial immunity, but in others the variation may be due to racial differences in sanitary habits.

It is general knowledge that some families or members of a family contract all the communicable diseases with which they come in contact, whereas other individuals of the same family or race escape. When a town's water supply becomes infected some individuals contract disease while others escape. This is known as individual immunity. Laboratory animals, rabbits guinea-pigs, etc., on which most experiments are conducted, show

very slight individual variation. In fact, the astonishing uniformity of reaction on the part of guinea-pigs of similar age and weight against measured quantities of bacterial toxins makes it possible to use these animals to standardize antitoxins. This has led some workers to believe that there is no individual immunity; what is claimed by some as natural individual immunity they believe is really an acquired immunity, and probably due to an earlier slight unrecognized attack of the disease. This factor undoubtedly plays a prominent part in the so-called "individual immunity" to tuberculosis. Recent work has proved that immunity in the cases of dysentery and typhoid comes from a previous attack. It is quite likely that as the subject of immunity becomes better understood it will be found that the majority of all reported cases of individual immunity to the communicable diseases come from missed mild cases.

There are, however, clear-cut cases of individual immunity due to age. There are certain diseases, commonly classed as children's diseases, to which the individual shows greater immunity at one age than another: Ringworm, diphtheria, and scarlet fever are among these.

Acquired Immunity.—Acquired immunity is the outcome of a successful combat between the host and the invading microbe. There has been produced within the body of the invaded, antibodies which are inimical to the microbe. Where the immunity has been attained as the result of an attack of the disease itself, it is referred to as "natural or spontaneously acquired immunity." When produced with some form of treatment, the products of micro-organism or the organisms so altered as to give a mild attack, it is spoken of as "artificially acquired immunity." The immunities resulting from vaccination for smallpox and typhoid are examples of artificially acquired immunity.

Active and Passive Immunity.—Active immunity is always due to the reaction of the individual's own body tissues against micro-organisms (living or dead), or their products (toxins or the poisonous products of their bodies). The substances which protect the individual are manufactured in his own body. Passive immunity is always brought about by the introduction of blood or serum from another animal which has previously developed an active immunity. That is, in passive immunity the defensive substances are produced in one animal and then transferred to the one which is to be protected.

Active immunity results from a mild attack of the specific disease in the individual who is to be protected; hence, the pro-

tection is nearly the same as that which comes from a natural attack of the disease. Immunity develops some time after the micro-organism, or its product (the antigen), has been introduced into the body of the individual. There is a reaction on the part of the individual. There usually is slight illness, and certain tissue changes occur during the time the substances, the antibodies, are being developed. The body has learned how to build its own antibodies; hence, the immunity is lasting. In passive immunity the greatest resistance occurs immediately after the serum has been given the individual. There is no negative phase. No illness results from the introduction of the antibodies. The antibodies are foreign substances and are rather quickly disposed of by the body. In passive immunity, the body has not learned to produce its own antibodies; hence, immunity is short-lived.

Early Attempts at Immunization.—It is quite generally known that one attack of some of the communicable diseases insures the individual against any further attacks. Among the diseases which insure lasting immunity after one attack are the following:

Cholera.
Plague.
Yellow fever.
Typhus fever.
Poliomyelitis.
Smallpox—second attack very rare.
Chickenpox—second attack very rare.
Mumps—second attack very rare.
Scarlet fever—second attack about 0.7 per cent.
Measles—second attack uncommon.

In other diseases, such as pneumonia, gonorrhea, and tetanus, there is no lasting immunity regardless of the number of attacks an individual may have previously suffered.

These facts were observed and commented upon by ancient writers, some even suggesting that it might be possible to produce an artificial immunity. It is stated that Mithridates the Great (132 to 65 B. C.) attempted to secure immunity against certain poisons by subjecting himself to increasing doses of poisons. His "antidote" contained sixty-four ingredients, some of which were used in infinitesimal amount and "mixed with the blood of Pontic ducks because they lived on poisons." The ancient inhabitant of China and India not only observed this phenomenon, but actually made use of it. They inoculated children with smallpox from active pustules, or had patients

sleep in their beds, or wear the shirts of sufferers. These methods were dangerous, but there usually resulted a milder case than those contracted in the ordinary manner.

Modern Methods of Immunization.—Pasteur, during the last phase of his life activity, concentrated on the prevention of disease by inoculation with attenuated viruses. His brilliant and successful attacks on chicken cholera, swine erysipelas, anthrax, and rabies created a new faith, which has been ably sustained by later investigators: That man will some day conquer the communicable diseases. Although this work has developed along many diverse and intricate lines and has reached a magnitude that challenges the most brilliant minds, yet the methods used for producing artificial immunity may be summarized under these five heads:

I. *By the Use of Attenuated Cultures.*—Attenuation may be accomplished in various ways: (1) The virus may be grown in the body of a less favorable host. Smallpox virus is attenuated by growing in the body of the cow, and Behring attempted to immunize cattle by use of the human type of *Mycobacterium tuberculosis*. (2) The organism may be grown under unfavorable laboratory conditions, such as too high a temperature or unfavorable nutritive media. Pasteur grew anthrax bacilli at 42° to 43° C. and then used them as a vaccine against anthrax. (3) The organism may be grown in a suitable medium and then subjected to adverse conditions, such as drying, chilling, or the addition of some chemical. This is exemplified in Pasteur's treatment for rabies. Germs weakened in this manner are very unlikely to cause serious attacks of their respective diseases. This method has proved most successful with the filtrable viruses. Where vaccines contain attenuated bacteria their use is confined largely to veterinary medicine.

II. *By the Use of Killed Cultures.*—The micro-organisms may be killed in various ways: (1) Heat, this is the method used in preparing vaccines against typhoid, cholera, and dysentery. The typhoid bacilli are heated to 56° C. for several hours. The temperature employed should be as low as will yield a sterile vaccine. High temperatures impair the antigenic properties of the vaccine. (2) Chemicals, such as thymol, chloroform, ether, acetone, carbolic acid, tricresol, and formalin have all been used. Probably best results have been obtained with formalin, which yields a sterile vaccine and preserves its antigenic properties for as long as one year. This is being used in the preparation of pneumococcic vaccine. (3) The organisms may be killed by

alternate freezing and thawing or by letting the cultures autolyze, after which the proteinaceous substances are extracted and used as the antigen. This was the method used by Koch in his unsuccessful attempt to immunize against tuberculosis. (4) Mechanical disintegration of the bacteria. In the light of modern knowledge concerning antigens, Kreuger has introduced what appears to be a rational method for the preparation of vaccines. The bacteria are grown on an appropriate media and the dense suspension thoroughly disintegrated under aseptic conditions in a mechanical grinder, after which they are filtered through an acetic-collodion membrane, tested for sterility and then used as a vaccine.

III. *By the Use of Toxin-antitoxin.*—The bacteria are grown under suitable conditions, the toxin separated and standardized on susceptible animals, and used mixed with an appropriate quantity of antitoxin. In the past, this has been the main method used in immunizing against diphtheria.

IV. *By the Use of Toxoid (Anatoxin).*—When a toxin is heated, exposed to ultraviolet light, or treated with chemicals it loses part, or all, of its toxic properties but retains its property of uniting with antitoxin, and if given to suitable animals calls out "antitoxin." Such a modified toxin is called toxoid or anatoxin. Diphtheria toxoid, prepared by the action of formalin on diphtheria toxin, is being used with great success in protecting against diphtheria.

V. *By the Use of Antitoxin.*—When toxins are injected in susceptible animals in small and increasing doses, there appears in the blood antitoxins. The blood serum of the immunized animal may be injected into a second animal, rendering it immune. This method is used in immunizing against diphtheria, tetanus, botulism, and scarlet fever. The resulting immunity is short and is spoken of as passive immunity, whereas each of the other methods yield an active immunity.

Antigens.—Those substances which, when introduced into the body without digestion, give rise to specific alterations in the reactivity of the tissue cells of the treated animals, are antigens. When administered in a manner preventing their digestion, they call out "antibodies" and possess the power of combining with them. It is Nature's method of dealing with a foreign protein which has accidentally entered the tissue. All complete naturally occurring proteins, whether of plant or animal origin, possess antigenic functions. Hence, all bacteria, yeasts, and molds, even though they are not pathogenic, possess this quality. Anti-

gens are complex colloidal substances consisting of two parts, one of which is a large colloidal particle which cannot diffuse into the cell; hence, Nature manufactures emergency substances, antibodies, to deal with them. Attached to these colloidal particles is a second group, Landsteiner's haptines. They are usually polysaccharids and give to the antigens their specificity. All true antigens possess them whether they be bacteria, yeast, molds, red blood cells, or blood proteins. Hence, an animal receiving antigen, for instance red blood cells from the horse, produces antibodies which are specific and in this case react only against red blood cells of the horse. The haptines are rather easily changed by heat, light, shaking, and chemicals. Hence, when micro-organisms are drastically treated, as in some of the earlier methods used in preparing vaccines, they lose their power of calling out specific antibodies. This is one of the reasons why many bacterial vaccines have been found valueless. In recent methods of preparing vaccines the antigens are treated only sufficiently to insure a sterile vaccine.

Reactions of the Body to Bacteria.—The reactions which occur in the body during an invasion by micro-organisms vary with different diseases. In some, there is a destruction of the microbes by the white corpuscles; in others, certain soluble substances are produced which inactivate and eventually kill the germs. In still others, substances are formed which neutralize the poisonous products. In most diseases a number of different weapons are used against the invader. Broadhurst tabulates the various reactions as follows:

- Reaction to infection
  - Increase in white corpuscles
  - Production of antibodies
    - Against toxin (Antitoxin)
    - Against bacteria
      - Acting alone—dissolving bacteria (lysins)
      - Acting with white corpuscles
        - Clumping bacteria (Agglutinins) and Precipitins
        - Digesting bacteria (Opsonins)

White Corpuscles.—The white corpuscles are colorless, nucleated cells extremely variable in shape and number. The blood of a normal adult contains from 7000 to 9000 per cubic millim-

eter. They are of several varieties and may be classified as follows: (1) *Lymphocytes,* which are about the same size as the red corpuscles and contain large spherical nuclei filling almost the entire cell. They comprise about 20 per cent of the white cells. (2) *Mononuclear leukocytes,* comprising from 2 to 4 per cent of the white cells. They resemble the large lymphocytes. (3) *Transitional leukocytes,* which are about the same size as the large lymphocytes with a nucleus that is horseshoe shaped. They comprise from 2 to 4 per cent of the white cells. (4) *Polymorphonuclear leukocytes,* which comprise about 70 per cent of the white cells. Their nuclei are irregular in shape, usually appearing in three lobulations and sometimes four. Three types of this group are recognized, depending upon their staining.

The white cells protect the body against foreign substances. They possess independent movement; hence, can gather at the site of threatened injury. They can even pass from the blood vessels into the surrounding tissue, where they pick up and digest bacteria or other foreign material with which they come in contact. This activity is called phagocytosis and the cells are known as phagocytes. Most of the ingestion and destruction of bacteria is done by the polymorphonuclear leukocytes. "They are police, judge, jury, and jail all in one. They arrest, engulf, digest, and destroy the criminal germs. If microbic outlaws enter the blood stream, these leukocytes march upon and annihilate them. If the germs enter the tissue the white blood

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Fig. 108.

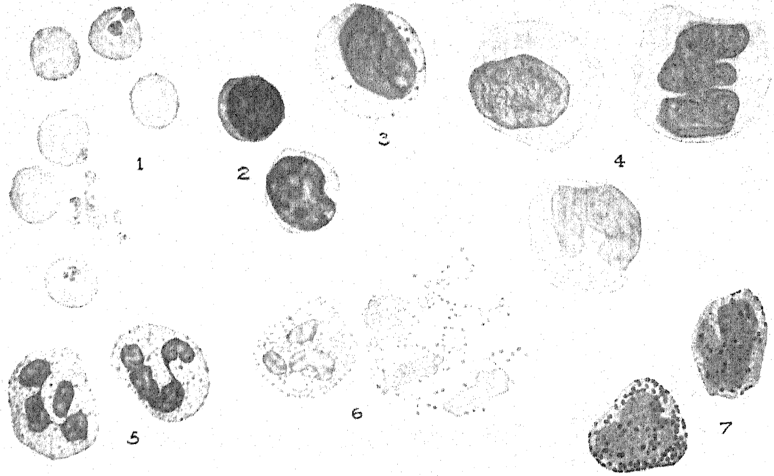
Fig. 1.—The cells of normal blood reproduced from actual cells. Wright's stain (×1000: 1 mm. = 1 μ).

1, Red corpuscles and blood-platelets. 2, Two lymphocytes. 3, A lymphocyte with azurophilic granules. This cell lay in a thin portion of a film and was exceptionally large. 4, Three endothelial leukocytes, one with fine cytoplasmic granules. The granules are rarely so distinct as here shown. 5, Polymorphonuclear neutrophils. 6, Eosinophils, one ruptured. The cells selected for drawing contained fewer granules than are usual. 7, Basophils.

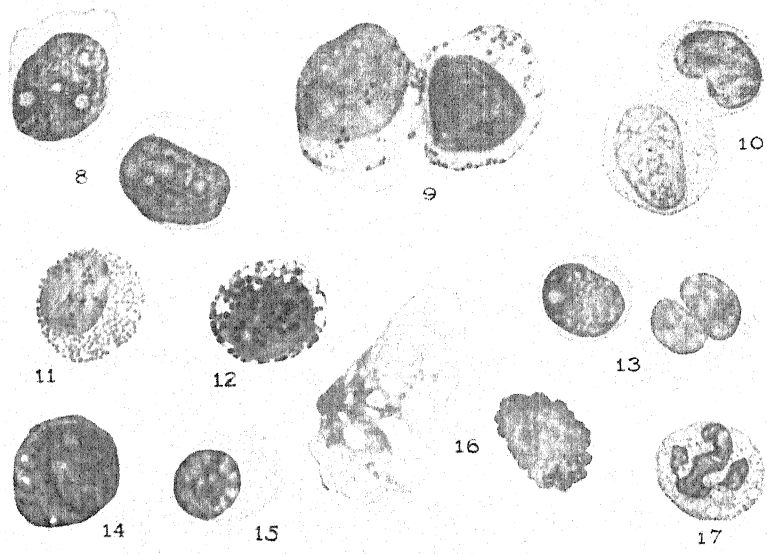
Fig. 2.—Leukocytes found in the blood in disease. All reproduced from actual cells stained with Wright's stain, exception No. 15, which is copied from Pappenheim (×1000: 1 mm. = 1 μ).

8, Two myeloblasts, showing nucleoli. 9, Two young myelocytes. Note the blue edge of one. 10, Two mature neutrophilic myelocytes. 11, Eosinophilic myelocyte. 12, Basophilic myelocyte. Some of the granules have dissolved, leaving vacuoles and staining the cytoplasm. 13, Two lymphoblasts, one with lobulated nucleus (Rieder cell). Turck's irritation leukocyte with vacuoles. 15, Plasma cell. 16, Degenerated nuclei, one a so-called "basket cell." 17, Neutrophilic leukocyte with vacuoles.

(*Taken from Todd and Sanford, Clinical Diagnosis.*)



The cells of normal blood (scale: 1 mm. = 1 μ).



Leukocytes which appear in the blood in disease (scale: 1 mm. = 1 μ).

(J. W. Rennell, pinx.)

Fig. 108.—For description, see facing page.

cells swarm after them, and congregate around the alien horde in ever-increasing numbers. The felonious host is gradually surrounded, engulfed, and destroyed. The field of combat becomes strewn with the dead and dying corpses of microbes and leukocytes alike. In an ordinary infection, as a boil, the entire mass becomes partly softened and decomposed, necrotic, as the pathologists say, and eventually, forms that gruesome mass known as pus. When the boil is 'ripe'—that is, when the outlaw microbes are fairly under control—the evacuation of the pus usually leads to complete healing. Sometimes the outlaw germs prevail and cause widespread destruction in the body or even death; occasionally, the microbe settles down in a joint, or the heart, and keeps up a languid opposition to the efforts of the body to dislodge them. Usually, however, the leukocytes prevail and restore order."

The phagocytes play a predominating part in protecting us against the pyogenic bacteria; however, recovery from such diseases leaves no immunity as "the leukocytes that are veterans of one insurrection do not ordinarily survive long enough to participate in another. They are short lived. They leave no descendants."

Injury to the body cells, or infection by bacteria results in inflammation described as follows by Williams:

1. "The first stage is a preliminary contraction of the blood vessels of the part with a later dilatation and slowing of the blood current. The quantity of blood in the part is greatly increased, the small vessels becoming distended.

2. "In the course of an hour (as observed experimentally in the frog) the blood flow is slowed further, and the leukocytes increasing in great numbers in this area are arranged along the outer part of the blood stream, the so-called 'margination of the leukocytes.'

3. "Soon after the margination of the white cells there begins the passage through the walls of the blood vessels. This is the migration of the leukocytes. Reaching the area that has been irritated, these cells engulf irritating particles, or if the active agent is bacteria, they begin battle with these poisonous invaders.

4. "If the cause of the inflammation is bacterial the migrating leukocytes may completely overwhelm the invading organisms and remove the cause. In other cases where the bacteria are especially virulent or in exceedingly large numbers, many leukocytes will be killed in the process, with disintegration and dissolution of the dead cells, forming pus."

Toxins and Antitoxins.—Certain bacteria, such as those causing diphtheria, tetanus, botulism, scarlet fever, gas gangrene, and dysenteria elaborate powerful poisons, the toxicity of which varies with the different organisms. These are soluble and pass into the cultural media; hence, they are termed "soluble toxins" or "exotoxins." They are complex organic compounds, the properties of which have been considered on pages 137 to 139.

Behring and Kitasato, in connection with their early studies of diphtheria and tetanus toxins, learned that animals which had survived a series of injections of small quantities of these toxins had become highly resistant to them. They made a search for the cause and discovered in the blood serum of the resistant animals something which neutralized the toxin. This substance they called antitoxin. It circulates in the blood of the immune animals and immediately combines with its specific toxin and renders it harmless. Since that time other plant and animal toxins have been discovered, so that today we have antitoxins against snake venoms, scorpion, and spider toxins and the toxic compound obtained from the castor oil bean, ricin, in addition to others. They are complex substances, sensitive to heat, light, and oxygen, and form loose combinations with specific toxins, thereby preventing the latter from injuring the animals.

They are standardized by determining the quantity necessary to neutralize one minimum lethal dose (M.L.D.) of a toxin. A minimum lethal dose of a toxin is the amount of toxin which, when subcutaneously injected, causes death of a 250 Gm. guinea-pig in from four to five days. A unit of antitoxin is approximately the quantity which will neutralize 100 minimum lethal doses.

When heated, or treated with appropriate chemicals, toxins lose their power of injuring but retain their property of calling out antitoxins when administered to suitable animals. Such modified toxins are called toxoids. To explain this condition, Ehrlich assumed that toxins have two essential parts: (1) One, which combines with the cell receptor, he designated the haptophore portion; this is unharmed by heat, light or chemicals. (2) The other, which he named the toxophore portion, is easily destroyed by heat and light and is the portion that poisons the cell. Hence, a toxoid is a toxin in which the toxophore group has been destroyed and the haptophore group is intact and possesses the power of calling out antitoxin.

Reactions Against Bacteria.—Most bacteria do not produce true toxins; hence, they do not have the power of calling out

antitoxins. However, they do cause the production of other antibodies which destroy either the bacteria or their products. These are known as lysins, agglutinins, precipitins, and opsonins.

Lysins.—Nuttall demonstrated in 1886 that normal blood contains substances which act upon cells, causing them to become granular, then swollen, and finally completely dissolved. These substances are called lysins. Those which dissolve bacteria are known as bacteriolysins; those that dissolve red blood cells hemolysins; and those which dissolve epithelial, or other body cells, are known as cytolysins. These lysins dissolve foreign cells which enter the blood. This is not always a benefit, for they may in this manner liberate poisonous products. Moreover, their activity in the test tube is often greater than in the body. Nuttall, in one of his early experiments, showed that 1 cc. of

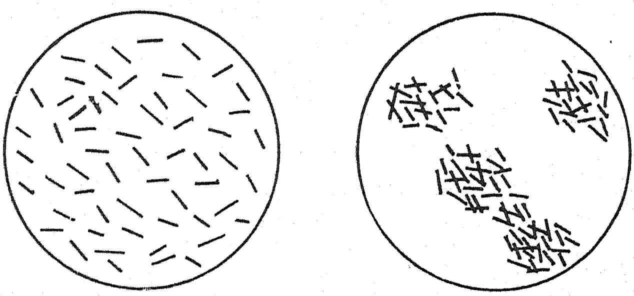


Fig. 109.—Illustrating microscopic agglutination reaction.

rabbit's blood contained sufficient bacteriolysin to destroy 50,000 anthrax bacteria, causing their complete destruction in four minutes; yet, according to some authorities 16,000 virulent bacilli will kill a rabbit when intravenously injected. The bacteriolysins are complex substances and readily destroyed by heat. They are specific.

Specific hemolysins may be produced by injecting red corpuscles from the blood of one animal into the circulation of a rabbit. The rabbit then produces hemolysins which react only with corpuscles from the same species as were those injected into the rabbit. That is, if human corpuscles were used to immunize the rabbit, the blood decomposes human corpuscles and no others. This phenomenon has found considerable practical application in the identification of blood and bacteria and in the diagnosis of syphilis.

Agglutinins.—When blood of a person immunized against a specific disease (for example, typhoid) is brought into contact with a suspension of the typhoid bacteria, it is found that all movement of the previously motile organisms ceases, and the bacteria stick together in small masses or clumps as if some sticky or glutenous substance were holding them together. This is spoken of as agglutination. It appears to be the preliminary change which occurs before the decomposition of the bacteria by the bacteriolysins. It is specific and, therefore, may be used for either the identification of unknown organisms, or the diagnosing of certain diseases and the finding of carriers. The individual who has typhoid fever will give a positive agglutination test when a drop of his blood is brought in contact with typhoid bacilli. This is known as the Widal test.

Precipitins.—Another class of immune bodies which may be produced in the blood are precipitins. They are produced when any foreign protein, bacterial or otherwise, reaches the blood stream. They are similar in most respects to the agglutinins. Some practical applications of the precipitin tests are: (1) The identification of blood. Blood stains are found on the clothing of a suspected individual. He claims they are from the lower animals. They are dissolved in a physiological salt solution and tested against serum from an animal sensitized to human blood. There results a precipitate. The evidence is that the stains were human blood. (2) Likewise tests may be made to identify certain proteins or to diagnose specific diseases. (3) The method may also be used to determine whether the blood of two individuals will mix, and consequently whether the one can be transfused into the other.

Opsonins.—If carefully washed phagocytes are brought in contact with bacteria they take up a few of the bacteria. If to a second batch of the phagocytes some normal blood serum is added and then this is allowed to act on bacteria, it will be noted that the phagocytes take up more bacteria. Now, if immune serum is added to a third batch of the phagocytes it will be found that this third group takes up still more bacteria. Hence, there is something in the blood of normal individuals in small quantities which make the bacteria more vulnerable to the attack of the white corpuscles. This something is increased in many infections and is known as opsonin.

Hypersensitiveness.—When an individual manifests greater response to a substance or condition than do other members of the species, that individual is referred to as being hypersensitive.

Some individuals are injured by contact with poison ivy or sumac, whereas others are not. Many develop hay fever or even asthma on inhaling certain plant pollens, but the great majority are unharmed. A small proportion of individuals become ill by eating what to the ordinary person is a valuable food. Sometimes the reaction toward the offending substance is extremely severe and may even cause death, under which conditions it is known as anaphylaxis. This is dramatically illustrated when a guinea-pig is given intravenously a foreign protein, for example, horse serum; after an interval of ten days the procedure is repeated. The animal appears normal for from one to three minutes, it then becomes uneasy, starts to rub its nose with its front paw. It may sneeze and occasionally cough. The animal may fall to its side, respiration becoming slower and shallower and even ceasing. The heart may continue to beat for a considerable time after breathing has ceased. After death the lungs are found to fill the whole thorax and many pathological lesions appear in the body. The symptoms are the same for a given species of animal, no matter what the foreign protein, but vary with different species. All natural occurring plant and animal proteins, including those from bacteria, yeast, and molds, are capable of giving the reaction. Extremely minute quantities will elicit the reaction, and there are cases on record in which one-millionth of a gram has given rise to a fatal anaphylactic shock. The reaction occurs in a modified form when an animal having tuberculosis or glanders is given a protein extract from the body of these bacilli. The reaction is specific and hence is the basis for the tuberculin and mallein tests used in the diagnosing of these diseases.

Theories of Immunity.—Various theories have been evolved to account for the way in which the body overcomes diseases. The two most generally accepted are the cellular and humoral theories. (1) The cellular or phagocytic theory of immunity was formulated by the noted Russian scientist, Metchnikoff, who observed in 1883 that certain cells of the body possess the power of ingesting and digesting bacteria. He and his followers concluded that these so-called "phagocytes" played the main rôle in the prevention of infection and in freeing the body of it. This theory explains very well the manner in which the body protects itself against many of the parasitic bacteria, but it falls far short of explaining the phenomena in the case of the progressive or true pathogens.

(2) In the humoral theory it is held that immunity is due to substances which result or are produced within the body, pri-

marily in the blood. This theory has been championed by Ehrlich and his followers and is usually referred to as Ehrlich's side-chain theory of immunity. It is based upon the observed power of tissues to regenerate themselves when injured and to produce an excess of tissue over that necessary to repair the wound. This excess, it is held, is thrown off and represents the various immune bodies which are observed and known to play a part in immunity.

The cellular theory of immunity was developed primarily by the French, and the humoral by the Germans. At first there was

Fig. 110.—Elie Metchnikoff (1845–1916).

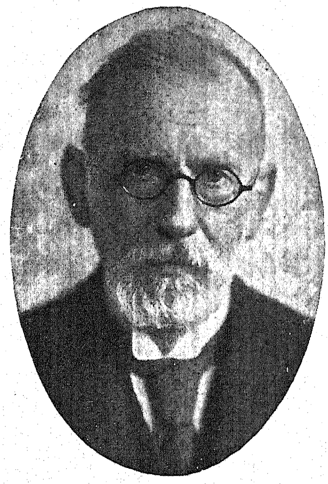


Fig. 111.—Paul Ehrlich (1854–1915).

considerable rivalry, each school trying to substantiate its pet theory, but now that the heat of the battle has cleared away, it can be seen that the facts point to the conclusion that in a measure both theories are correct and that immunity must be explained by a compromise or combination of the two theories. It is certain that the phagocytes can ingest and digest bacteria, and it is also true that there are certain substances, opsonins, produced within the blood which render the bacteria more vulnerable to attack. It is also clear that certain substances, antitoxins, are produced within the blood which neutralize bacterial poisons, and still other products, lysins, which decompose them.

CHAPTER XXXVI

THE BACTERIOPHAGE

THE space as well as the elements which are essential for the growth and normal functioning of plants and animals upon the surface of the earth is limited. Certain plants and most animals are so constituted that they must obtain their food in the form of definite compounds, which necessitates that they receive it in a prepared form from the bodies of other plants and animals. These conditions give rise to a constant struggle for existence. It manifests itself among the members of the same species; even man struggles with man for a place in the sun. Species compete with species, the higher preying upon the lower, on down the line. The little amebae devour the still smaller bacteria. Bacteria standing at the bottom return the compliment, on up the line with a vengeance, they being the greatest destroyers of all. They act as if they were the timekeepers in the race and fight of life, and stand as the link between the living and the dead. When the higher plants and animals have had their borrowed essential elements for their allotted time, the bacteria intervene, end the race, and return the elements to the great earthly reservoir, so that they can be used over and over in the never ceasing drama of life.

In this scheme of things it appears as if the bacteria stand apart from other forms of life, inasmuch as they have no smaller organisms to prey upon them. Due to their method of multiplication they never grow old; hence, barring accidents, they may be considered immortal. It is even argued that they were the first living organisms upon this planet, for some of them can subsist upon the inorganic constituents of the bleak rock watered with rain, receiving sufficient energy from the traces of ammonia which it contains. In the course of evolution, due to differentiation, the higher plants and animals became mortal, thus rendering them liable to natural death and also disease. While it is true that the single-celled forms of life never grow old and are, therefore, not subject to natural death, bacteria also may sicken and die, and as some argue, because of living parasites.

History.—Hankin, in 1896, found that the waters of India purify themselves. The Jumna River at Agra contained 100,000 bacteria per cubic centimeter, while 3½ miles below it contained only 90 bacteria. It is a well-known fact that this river is highly polluted, but the disease-causing bacteria quickly disappear from it. This is not due to a poison, for none is to be found in the water. The bactericidal substance is destroyed by heat, as cholera organisms quickly disappeared from the natural waters; yet they actually multiplied in the same water after it had been boiled.

Haffkine, who was in charge of a laboratory in which vaccine against the plague was produced, noted that some of his cultures of the plague bacteria would grow normally for a time, after which they would disintegrate and all the plague-producing organisms disappear. This was such a common occurrence that the workers gave to them the name of "suicides." Why did they commit suicide? If it was a suicide, what was the weapon used in self destruction?

The first explanation of this phenomenon was offered by Twort, who in 1915 was attempting to cultivate on agar ultramicroscopical organisms from the lymph of a calf. On some of his plates there appeared colonies of micrococci, and in some of these plates "watery looking areas." This apparent diseased condition Twort found could be communicated to other colonies. He made the following interesting suggestions concerning the etiology of this "disease" of bacteria: (1) That the active material might be produced by the micrococcus; (2) that the "virus" might be an ultramicroscopical organism, pathogenic for bacteria; (3) that it might be living protoplasm which formed no definite individuals; or (4) that it might be an enzyme with power of growth.

In 1916, d'Herelle, of the Pasteur Institute, had under observation an adult suffering with a severe case of dysentery. Each day a small amount of the feces was placed in a sterile tube of bouillon. After incubation at 37° C. over night, the growth was filtered through a clay filter which removed all the dysentery bacilli. This clear filtrate was added to a second tube to which had been added previously the dysentery bacilli. This was then incubated, and at the end of twenty-four hours the bouillon was cloudy because of the great number of bacteria which had grown within it. This procedure was repeated daily for some time, when one day, upon examining the tubes prepared the previous day, there was found to be no growth. When a drop of this was carried to another tube, it was found that growth

was prevented in this tube, even though it had previously been heavily seeded with the dysentery bacteria. After considerable work of this nature, d'Herelle suggested that there is in the filtrate a living "ultramicrobe" which develops as a parasite in the bacteria and destroys them. To this he gave the name bacteriophage.

Having learned that there is something which preys upon bacteria, many questions arose, such as: What is the bacteriophage? Where is it found? Is it possible to see it even with the ultramicroscope? Is it a living entity? If it is, what does it feed upon? Can they be counted? Are there different species or races? If so, do they all attack bacteria with the same vigor?

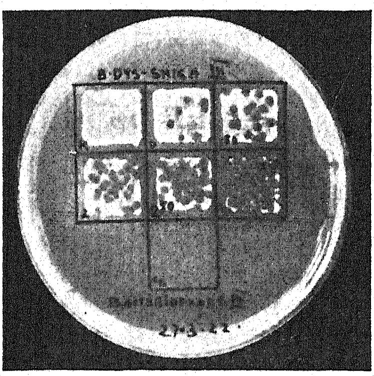


Fig. 112.—Bacteriophage acting on *Salmonella paratyphi β*. (After *Asheshow.*)

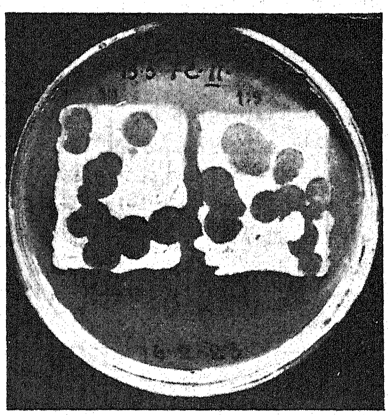


Fig. 113.—Bacteriophage acting on *Shigella dysenteriae* (Flexner). (*After Asheshow.*)

Can they be used by man to control the disease-producing microbes?

Source of the Bacteriophage.—D'Herelle first obtained the bacteriophage from the intestines; hence, he gave to it the name *Bacteriophagum intestinale,* and suggested that each new species be designated according to the bacterium on which it acts. Thus a "Shiga-bacteriophage" is one which was originally isolated from a case of dysentery due to *Shigella dysenteriae* (Shiga), or it is a bacteriophage virulent for this organism. A "staphylo-bacteriophage" was isolated from a lesion caused by the staphylococcus. A "cholera-bacteriophage" was originally obtained from a case of cholera.

The bacteriophage has been found rather widely distributed in nature. Its chief source, however, seems to be the intestinal tract of man and the higher animals. It is particularly abundant in individuals who are convalescent from a bacterial infection. It has also been obtained from diseased tissues, exudates, bacterial cultures, soil, water, and the like. Being a constant inhabitant of the intestines, it may be encountered in anything which is contaminated by human excreta.

Propagation of Bacteriophage.—The bacteriophage manifests itself only by its action on living bacteria. In reality, the only appropriate cultural media known for its propagation are living cultures of bacteria. So far it has been shown that it can be grown on some twenty different species, among which are both pathogens and saprophytes.

When once a sample of bacteriophage has been obtained, it may be propagated indefinitely by transferring small portions of the culture to a suitable medium containing young cultures of the appropriate bacteria. These must be living and should be young, as it does not multiply on dead bacteria. The medium may be either a liquid or solid, the only requisite being that it be suited to the growth of the specific bacterium which is used in conjunction with the bacteriophage.

If the bacteriophage is seeded into a liquid which is cloudy because of the presence of bacteria, the solution soon becomes clear. The bacteriophage digests completely the bodies of the bacteria, which then pass into solution. If it is seeded on to solid media heavily inoculated with some bacterium that is susceptible to the bacteriophage, it is found that no bacterial growth appears. If the bacteriophage is only lightly seeded, that is, only certain portions of the agar inoculated, these appear as small bare spots, "plaques," on which there is no bacterial growth.

Properties of Bacteriophage.—If a platinum inoculating needle is dipped into a "bacteriophage fluid" and the minute quantity adhering to the wire inoculated into a prepared bacterial suspension, the suspension becomes clear within a few hours; all of the bacteria have disappeared. To all appearances they have dissolved in the bouillon, just as sugar or salt dissolves in water. If a bit of this clear solution is transferred to a second suspension of bacteria, in a short time this also becomes clear. The bacteria have been digested. This procedure may be repeated a third, fourth, and any number of times, and the final results are always the same. By this method d'Herelle propagated bacteriophage for ten years, during which time it was transferred thousands of

times. It is not possible that there could have been anything in the original culture so injurious to bacteria that it could have been diluted to such an extent and still destroy bacteria. Hence, it is concluded that the bacteriophage multiplies.

Those who believe the bacteriophage to be a living entity follow the speed of multiplication and attempt to determine the number. The procedure is very similar to that used in counting bacteria. The number of bacteria in any substance is found by diluting with a measured volume of sterile water. The well-diluted material is poured into some sterile solid medium while in the liquid state. On solidifying, the bacteria become fixed on the surface and grow until they become so numerous that they may be seen with the naked eye—just as a clump of trees can be seen in the valley from the mountain side, whereas a single tree may be overlooked. Probably each group developed from a single organism; hence, if one knows the dilution he can calculate the number in the original sample after counting the colonies.

By seeding measured volumes of the substance to be studied and then pouring onto a solid medium heavily seeded with bacteria the bacteriophage can be counted. The bacteria, free from the bacteriophage, multiply and soon become visible, but where the bacteriophage is present the bacteria are prevented from growing; hence, we have a bare spot on the culture medium, and by counting the number of bare spots on the medium, one can ascertain the number of bacteriophage present.

Assuming that bacteriophage are living corpuscles, attempts have been made to measure their size. This is done by passing them through filters of which the size of the pores have been determined. If they pass through one filter of a definite size but not through another just a size smaller, it is known that in size they must be between the two. In this way it has been found that the bacteria are many thousand times larger than the bacteriophage. Bacteria are from 0.1 to 5 microns in length, the bacteriophage from 6 to 20 millimicrons. It requires five hundred million bacteria to weigh one milligram. It would require many thousand times this number of bacteriophage to weigh so much. It has been suggested that bacteria are as much larger than the bacteriophage as a fly is larger than bacteria.

These sizes approximate that of the protein molecule. It seems to leave us in a dilemma. Protein has been looked upon as the basis of life. Is it, thus, possible to have living organisms smaller than the protein molecule? An organism is considered a cell or an aggregate of cells, and a cell a mass of proto-

plasm containing a nucleus. When we learned of the bacterium we came to define a cell as a mass of protoplasm containing nuclear material. It may be that our conception of some cells will have to be modified to read: A cell may be a micella which is composed of material less complex than the protein. Moreover, it has recently been suggested that life may have originated with very simple compounds and not with the complex protein.

The bacteriophage possesses relatively high resistance to temperature; in some cases it is not inactivated when held at 70° C. for twenty minutes. Marked variations have been noted by different individuals. This has caused some to conclude that there is no definite thermal death point for the bacteriophage. D'Herelle explains it as due to different races. He further maintains that all races are completely inactivated in thirty minutes at 75° C. They are very resistant to low temperatures. They are destroyed by ultraviolet light and are sensitive to most chemicals including disinfectants but appear to be more resistant than are bacteria; hence, it is possible to destroy bacteria without inactivating the bacteriophage.

The bacteriophage may adapt itself to its surroundings. Just as Pasteur found it possible to increase the destructive powers of bacteria by successive passage through the bodies of susceptible animals, likewise d'Herelle finds it possible to increase the digesting power of the bacteriophage for bacteria by successive cultivation in the bodies of bacteria. By this means many races have been produced which quickly devour the typhoid, diphtheria, dysentery, and other disease producers. Moreover, these highly active strains are found in the bodies of individuals recovering from these diseases; whereas, when the disease terminates fatally, they are usually absent. Consequently, according to d'Herelle, they are often the determining factors in recovery.

Nature of the Bacteriophage.—Much discussion has centered around the nature of the bacteriophage and numerous theories and subtheories have been formulated. The chief of these are: (1) That the bacteriophage is a living, ultramicroscopic, filter-passing organism; the obligatory parasite of the bacteria. This theory is ably and ingeniously championed by d'Herelle and his followers. They point to the fact that it multiplies and can adapt itself to its environment. Moreover, a good deal of evidence goes to show that the material is a small particulate body for which he coins the term *"protobe."* (2) That it is the action of autolytic ferments. It is believed that these once introduced set going a series of autolytic processes in the bacteria

which result in their dissolution and the freeing of the lytic substance from their interior. In this way, once the necessary environmental conditions are established, the bacteria are unable to escape from it, and the process can therefore be carried on indefinitely. This theory, although championed by many, is somewhat shrouded in mystery and according to d'Herelle falls far short of accounting for the phenomenon. (3) A theory somewhat similar to the above is that propounded by Bordet, who believes there is a mutational change which occurs in bacteria which causes them to undergo autolysis, thus setting free an actively lytic substance which digests the bacteria. Probably the majority of bacteriologists subscribe to the two latter theories. However final judgment must await further discovery.

Bacteriophage in Immunity.—The discovery of the bacteriophage has caused d'Herelle to offer a modified theory of immunity into which three factors enter: (1) The bacteriophage; (2) cellular immunity; and (3) antitoxin immunity. The bacteriophage acts in three ways: (a) Through direct antagonism for the disease-producing agent; (b) by the preparation of bacteria for phagocytosis; and (c) by rendering the bacteria particularly potent as antigens. Thus, the bacteriophage plays a particularly important rôle, for, besides acting directly and independently against the pathogenic agent, it is indirectly concerned in the other two factors. A concrete case will help to give a clear conception of the theory. When typhoid bacilli succeed in entering our tissue, one of four things follows. (1) If we are fortunate enough to be carrying a bacteriophage virulent for the typhoid bacilli, the invading organisms will be attacked and destroyed by it before they have had time to multiply and cause an infection. In this case no disturbance arises, and we remain unaware of the coming and going of the bacilli; theoretically, a degree of immunity would arise as a result of the action of antigenic substances derived from the destroyed bacilli. (2) We may be carrying a bacteriophage that is not actively virulent for the invading bacteria which, unopposed, give rise to infection and disease. In the meantime, the bacteriophage acquires virulence for the infecting bacteria and destroys them before the host is overwhelmed. The disease is thus checked and the patient recovers. (3) The bacteriophage may be able to oppose the bacilli sufficiently to prevent disease but not to destroy them. It is thus that typhoid carriers arise. (4) If the bacteriophage is absent, or present and unable to interfere with the growth of the bacilli, a fatal infection develops. D'Herelle even believes "that the rise

and decline of epidemics is due to the swaying battle between the bacteriophage and infecting bacteria and that, with the establishment and diffusion of a bacteriophage of sufficient activity, an epidemic is normally brought to an end."

The Bacteriophage as a Preventive and Curative Agent.—The bacteriophage has been extensively used by d'Herelle in the cure and prevention of numerous bacterial diseases in man and the lower animals. Many of his cures have been little short of marvelous. This has caused some to ask: Will the bacteriophage eventually be developed so that it can be liberated in water and thus free waters of harmful bacteria? Will the physician of the future combat the communicable diseases of man by the use of virulent bacteriophage which will prey upon the disease-causing bacteria? Will it be possible to make immunity "catching" as is now the case with disease? The claims made by d'Herelle are that we shall. This sounds "too good to be true." Moreover, most workers have failed to confirm his findings. Future work must solve this riddle.

CHAPTER XXXVII

ANTITOXINS AND VACCINES

MICROBIAL infection is nothing more or less than an immunizing contest between a host and an invader. Each contestant is feverishly engaged in manufacturing products with which to destroy its opponent or to render itself immune against the weapons of its enemy. Hence, the outcome of the struggle is determined by the quality and quantity of the defensive and offensive products produced by the contestants. Any advantage which one can gain over the other often determines the outcome. Man is learning how to supply the host with defensive weapons in the form of antitoxins and how to stimulate the cells of the host by vaccines to a rapid production of both offensive and defensive weapons before he is attacked by his microbial enemy.

Historical.—It was Frederick Löffler, a student of Koch, who discovered the bacillus which causes diphtheria, and Emile Roux, a pupil of Pasteur, who demonstrated that in this disease the organism grows at the site of inoculation and elaborates a toxin which is distributed by the blood and injures the tissues. It was found that by growing some of the bacteria in bouillon, filtering off the germs, and injecting the clear filtrate into the tissues of susceptible animals, diphtheria resulted. Through further experimentation on guinea-pigs another student of Koch, Emil Behring, succeeded in showing that recovery from the disease is due to the production of antitoxin which counteracts the poison in the blood. By 1895 Behring and others had so far perfected a method of producing antitoxin on a large scale that it could be used in the treatment of diphtheria. From that day to this the diphtheria death rate has steadily declined. The diphtheria rate per 100,000 population has dropped in New York City from 110 in 1895 to 10 in 1925. During the same period the mortality has decreased from 35 to 7, and of those who receive antitoxin treatment on the first day of their illness the mortality is less than 1 per cent.

Nature of Antitoxins.—Antitoxins are antibodies capable of directly and specifically neutralizing toxins that cause their production. There are both animals and plants which produce

toxic substances possessing the power of calling out antitoxins. The venom of the rattlesnake and cobra, the poison of the scorpion and the tarantula, the poisons produced by certain fishes and insects are examples of animal toxins. They are known as zootoxins. Toxins produced by plants (phytotoxins) are found in the juice of the castor oil bean, ricin, the bark of the locust tree, robin, poisonous mushrooms, and many other plants. Only a few bacteria produce true toxins. The principal ones are the diphtheria, the tetanus, and the botulinus bacilli, and probably certain forms of the dysentery bacilli and *Pseudomonas aeruginosa*.

Antitoxins are less resistant to heat and chemicals than are most toxins which cause their production, boiling, putrefaction, and many chemicals destroy them. They are complex organic compounds associated with the serum globulin and are possibly proteinaceous in composition. All attempts to separate them from the blood proteins have so far failed. Hence, a more accurate designation of the biological product is antitoxic sera and not just antitoxin. In the serum of the blood of animals immunized to the true toxins the main defensive product is antitoxin. There are usually present other antibodies, lysins, opsonins, precipitins, and agglutinins, which assist in overcoming the invading organism. Antitoxins are recognized by their power of neutralizing toxins with which they apparently combine and render nontoxic. They are produced in the bodies of animals, as a result of the entrance of a toxin; consequently, each antitoxin has a corresponding toxin that causes its production.

Inasmuch as many individuals fail to produce the appropriate antitoxins or else produce them in inadequate quantities (and hence would die or suffer irreparable damage from the attack), modern therapeutics forestall these failures on the patient's part by supplying him with the antidote from another source. That is, the patient is given an antitoxic serum which contains the appropriate antitoxin for neutralizing the toxin produced by the invading organism. In other cases the cells of the individual are trained beforehand to produce the needed defensive substances by the use of an appropriate vaccine. The latter is nothing more or less than a mild attack of the disease.

Preparation of Diphtheria Antitoxin.—Diphtheria and tetanus antitoxins are manufactured today on a large scale and are extensively used in the prevention and cure of these diseases. But, inasmuch as antitoxins are produced in the body of an

animal only by the stimulation of a toxin and then transferred to a second animal, it is necessary first to consider the preparation of the toxin.

Toxin Production—Diphtheria toxin is produced by seeding the germ in large flat-bottom flasks containing a relatively thin layer of a specially prepared nutritive broth. For the best results the media must be prepared with great care from fresh lean beef which has been freed from muscle sugar by fermentation with *Escherichia coli.* It is essential that the diphtheria organisms receive an abundance of oxygen and be grown at a temperature of from 35° to 36° C. for maximum toxin production. The growth spreads over the surface of the broth, and in from seven to ten days the container is removed from the incubator, tested microscopically for purity, and a little carbolic acid added to kill the bacteria. After forty-eight hours the dead bacilli have settled to the bottom. The clear liquid is then siphoned off and filtered through a porcelain filter to remove any bacterial bodies which may be present. The strength of the toxin is determined by injecting carefully measured quantities into guinea-pigs weighing 250 Gm. The smallest quantity that is sufficient to cause the death of such a test animal in a given time is taken as the unit and is known as the minimum lethal dose, "M.L.D." Knowing this, the manufacturer of antitoxin can ascertain with certainty how much may be used with safety on the animal which is to be used for the preparation of antitoxins.

Preparation of Diphtheria Antitoxin.—In the preparation of diphtheria antitoxins the horse is used. This animal is used in this connection for several reasons: (1) It produces large quantities of antitoxin. (2) Because of its size, considerable quantities of blood may be taken without permanent injury. (3) It produces a blood, the proteins of which are borne better by man than is the blood from other appropriate animals. Young, vigorous, absolutely healthy horses of fair size are used. Every precaution is observed to see that they are free from disease. Generally, it is the practice to give first some antitoxin, and on the following day to inject a small quantity of toxin under the skin. Usually for a day or so the horse refuses to eat and there is some fever. As soon as the fever subsides, usually in two or three days, a second and larger dose of toxin is given. This is continued for 7 or 8 doses, each dose being slightly larger than the preceding one. Smaller quantities are then given until the maximum antitoxic content of the blood is

reached, which occurs only after several months and can be determined by bleeding the horse and testing the blood against a standard toxin.

There is a wide variation in the resistance of horses to toxin. Some horses may be seriously injured or even killed by 0.01 cc. of an ordinary toxin whereas others may receive 10 cc. of the same toxin without injury. The latter naturally carry antitoxin in their blood and are better suited for the production of antitoxin than are ordinary horses. When a horse is found which has the power of producing a highly potent serum, it is used for long periods for the production of antitoxin.

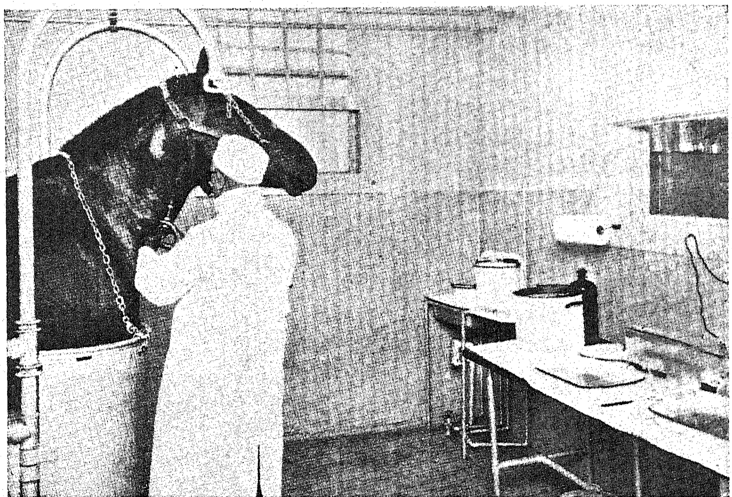


Fig. 114.—Injecting serum horses. (*Courtesy of Parke, Davis and Co.*)

When the blood of the horse reaches a high antitoxic content, the horse is bled by introducing a hollow needle, or cannula, into the right jugular vein, and the blood is allowed to flow into a sterile jar. The cannula, tubing, jar, and everything used in collecting the blood are carefully sterilized, and the whole operation is conducted with the greatest of aseptic care so as to avoid contamination. From 6 to 12 liters of blood are removed. The horse is then permitted to regain its strength after which the injection of toxin is again commenced, and after a time the bleeding is repeated. In a recently proposed method, the blood is collected into an oxalate solution, the corpuscles separated from the plasma and the corpuscles reinjected into the horse.

This prevents anemia and other bad effects which follow the too frequent removal of large quantities of blood.

The jars containing the blood are placed in a refrigerator for a few days, during which time the blood clots, and the clear serum collects on the surface. The latter is siphoned off and passed through porcelain filters to free from any solid particles including bacteria which may have gained access to it. The serum should be clear and free from blood. Its sterility is determined by microscopical and cultural tests while its strength is determined by mixing accurately measured quantities with a toxin of known strength and injecting this into guinea-pigs of

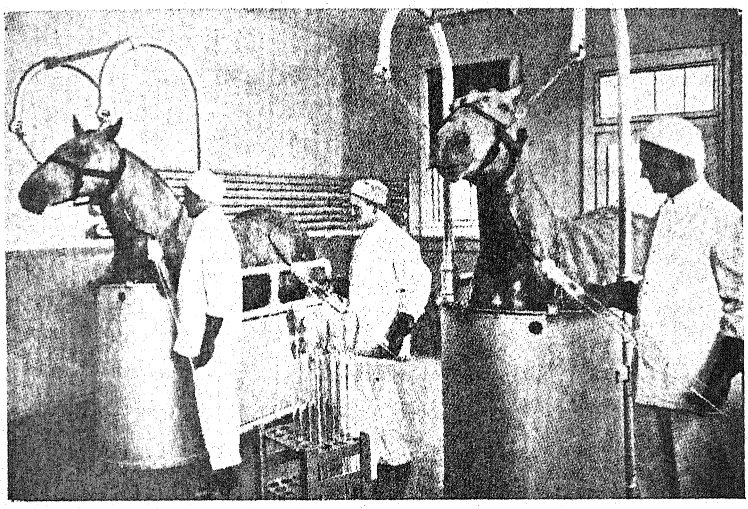


Fig. 115.—Bleeding antitoxin horses. (*Courtesy of Parke, Davis and Co.*)

standard weight. Often the antitoxin is concentrated by definite chemical procedure before standardizing, so as to remove much of the objectionable protein which tends to cause serum sickness in the recipient. When the serum is to be kept for some time a small quantity of an antiseptic is added, after which it is dispensed in receptacles, usually closed syringes, ready for use. It deteriorates more rapidly at high than at low temperatures; hence, it should be stored at low temperature. Most laboratories place the date after which it should not be used on the label. Not that it becomes injurious on standing, but it loses strength to such an extent that after a time it cannot be depended upon as a curative or preventive agent.

Antitoxin is used extensively as a curative agent, and if given to the patient on the first day of the disease usually effects a cure. Later use is not as successful, for the diphtheria toxin rapidly enters the tissue and does its damage. Where paralysis follows a case of diphtheria in which the antitoxin has been given, the injury results from the disease and not from the antitoxin. The antitoxin may have been given too late or in too small a quantity; consequently, the toxin was not neutralized. The antitoxin itself is harmless. In some cases serum sickness follows the injection of the foreign protein into the blood stream. This is usually mild and caused by the blood proteins and not by the antitoxin. Antitoxin is also used as a preventive where individuals have recently been exposed to diphtheria.

Toxin-antitoxin and Anatoxin.—It was Behring who first used toxin-antitoxin to immunize against diphtheria, but it was Park who made of it an effective weapon against diphtheria. He prepares toxin-antitoxin by mixing the two in proportions so that each cubic centimeter contains $\frac{1}{10}$ L. of toxin + $\frac{3}{40}$ unit of antitoxin. Antitoxin was obtained from the horse at first but later from sheep or goats so as not to sensitize the recipient to horse serum. Three doses of 1 cc. each are given subcutaneously at intervals of seven days. The symptoms are mild, and there results in from three to twelve weeks an immunity which may last for life. The actual duration of this immunity can be determined by the Schick test.

Ramon's anatoxin is coming into favor within recent years, and it is probable that before long it will replace toxin-antitoxin. Anatoxin or toxoid, as it is sometimes called, is prepared by mixing toxin with 0.4 per cent formalin and storing for a month or more at a temperature of 37° to 40° C. It has the advantage over toxin-antitoxin in that: (1) It is less toxic and hence can be used with absolute safety; thermostable toxin-antitoxin solutions have been known to become toxic due to freezing and the improper mixing of the toxic antitoxin; (2) stable anatoxin has been kept for seven years without deterioration; (3) irreversible; the toxoid having once been produced from the toxin cannot be reconverted into toxin; and (4) it is just as effective in producing an immunity of long duration as is toxin-antitoxin.

Other Antitoxins.—When the great curative and preventive value of diphtheria antitoxin was discovered great hopes were entertained that the same principle could be applied to other diseases, and we only needed to await the time when the millenial hope of a diseaseless world would occur. However, this hope

has been satisfied in few other cases. Diphtheria antitoxin is still the outstanding example of a real curative serum. Tetanus antitoxin is the only other antitoxic serum which even approaches this hope. It has high protective but low curative value. Antitoxins have been prepared capable of neutralizing the toxin of the botulinus organism and snake venoms. These are manufactured in essentially the same manner as the diphtheria antitoxin.

Vaccines.—A vaccine is an attenuated or killed suspension of the organism inoculated into the body to produce active immunity. When it consists of a suspension of killed bacteria, it is usually referred to as a bacterin. Ordinarily, the bacteria are killed; occasionally, they are introduced into the body by an unusual portal. In any case, they produce only a mild form of the disease which is followed by a degree of resistance, varying with the specific micro-organism and the treatment it has received.

When Jenner took cowpox material from the hand of the milkmaid, Sarah Nelmes, and put it into the arm of the eight-year-old James Phipps, he laid the cornerstone of a new edifice in preventive medicine, the use of vaccines in the control of diseases. Pasteur's contributions were the use of attenuated viruses against fowl cholera, anthrax, swine erysipelas, and rabies. His followers have conquered typhoid, plague, and cholera with killed bacilli, and today a host of able workers are feverishly endeavoring to perfect vaccines against pneumonia, whooping cough, poliomyelitis, tuberculosis, and many other diseases, with promise of success in the new light of our present knowledge of antigens.

Smallpox Vaccine.—The first, and most successful use of a vaccine for the prevention of a communicable disease, was in the case of smallpox. The term "vaccine" originated from the term *vacca,* meaning a cow, but it is now used in a broader sense. In the early days of smallpox vaccination it was the custom to vaccinate one individual from the pustule on the arm of another. The old-time physicians saved the scabs from typical vaccination cases and carried them about in suitable containers ready to vaccinate their patients. There are two great objections to this method: (1) The supply was limited and was collected by various individuals without proper sanitary care. (2) It was possible in this way to spread human diseases, especially syphilis.

For many years it has been the custom to propagate the vac-

cine in young healthy animals. This obviates all danger of human disease and makes it possible to prepare a uniform vaccine under carefully controlled sanitary conditions. Various animals have been used. Female calves from two to four months of age are preferred, because they are easily managed and have such a tender skin that the eruption formed on it is abundant and typical. Furthermore, they can be kept on a milk diet, thus obviating the dust which is associated with the use of fodders. The animals are held under observation in quarantine for seven days to determine the presence of any infectious disease and are then thoroughly cleaned before vaccinating.

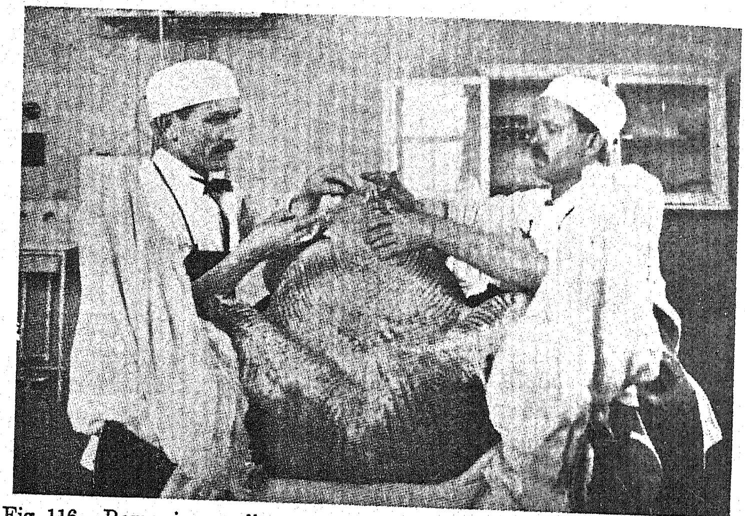


Fig. 116.—Removing smallpox vaccine. (*Courtesy of Parke, Davis and Co.*)

The belly and flanks are clipped, and the skin is thoroughly washed with soap and sterile water. Each animal is then vaccinated by making about 100 small scarifications and carefully inoculating these with the seed vaccine which has been prepared in the following manner: "Crusts are collected from healthy children about nineteen days after successful vaccination. These crusts are cut up and emulsified with boiled water to a mucilaginous paste. This humanized seed is inoculated into an area about 6 inches square upon the abdomen of a calf, the remainder of the calf being vaccinated in the ordinary way. The pulp from this special area is separately collected and glycerinized in the usual way. It is then tested bacteriologically and clinically.

This bovine virus from human seed is now used in a dilution of 1 part to 12½ parts of normal salt solution to vaccinate rabbits. The seed is rubbed thoroughly on the freshly shaved skin of the back. Five days after vaccination the pulp is removed with a curet, weighed, and emulsified in a mortar with the following solution: Glycerine 50 per cent, sterile water 49.5 per cent, and carbolic acid 0.5 per cent, in the proportion of 1 part of pulp to 8 parts of the solution. Four rabbits should yield from 15 to 20 cc. of this emulsion, an amount sufficient to vaccinate one calf."

The animals are kept in clean stables, and in five or six days the pustules which have formed at the site of the vaccination are ready for the collection of the virus. The calf is killed, the vaccinated area thoroughly cleaned, and the vaccine collected under as rigid aseptic conditions as are used in a major operation. The vaccine is mixed with glycerin and allowed to stand for three or four weeks so as to destroy bacteria which may have gained entrance. Its strength is measured by inoculating into rabbits. Its bacterial content is determined by plating and inoculating into guinea-pigs. The calf from which the vaccine was collected is carefully examined by a veterinarian, and if any signs of disease are found the entire batch is destroyed. After it has met all careful laboratory tests, and not until then the vaccine is submitted to a final clinical test on unvaccinated individuals, after which it is placed in fine tubes, or on points and is ready for distribution. The yield of vaccine varies greatly with calves; some yield only sufficient for 500 vaccinations, whereas others yield sufficient for 10,000. It should be kept at a low temperature (35° to 40° F.). Often failures in vaccinating individuals successfully are due to the deterioration of the virus which may have been kept in a warm place. From what has been stated it may be seen that every precaution known to man is used to insure a safe reliable vaccine, with the result that it can be used knowing that one is endangering neither life, nor limb, and that it will absolutely prevent smallpox epidemics.

Rabies.—This is a disease transmitted by the bite of a rabid animal. Until the time of Pasteur it had a mortality of practically 100 per cent. Today when properly treated it has a mortality of less than 1 per cent. However, to be successful, the Pasteur treatment must be given before the onset of the symptoms. The virus is usually attenuated by drying and less often with chemicals.

Pasteur found it necessary first to obtain a fixed virus. This

he did by taking some of the nervous tissue of a rabid dog and injecting it into a rabbit. Nerve tissue is obtained from this rabbit and inoculated into a second and so on until it has passed through some 30 to 50 rabbits. By this time the virus has been so intensified that it kills a rabbit in from six to eight days. This is referred to as a fixed virus and is inoculated into another rabbit. This animal is kept under the best of sanitary conditions and just before its death is chloroformed, and the spinal cord removed under aseptic conditions. The cord is cut into pieces, and dried over an appropriate drying agent—parts of it for one, other parts for three, and so on up to eighteeen days, after which it is mixed with water and given hypodermically to the patient. The pieces of the cord which have been dried longest, from eight to eighteen days, depending upon the method used, are given first, followed at daily intervals by the cord which has been dried for a shorter time, so that eventually the individual is receiving cord which has been dried for only one day.

Typhoid Vaccine.—In vaccination against typhoid the bodies of the dead bacteria are used. The organisms are grown on agar, and after twenty-four hours are washed with sterile normal salt solution, standardized by counting the number of organisms in a given quantity, and then killed by heating to 56° C. for one hour. As a matter of safety 0.25 per cent of tricresol is mixed with it. In order to immunize individuals against typhoid fever, three injections are given at intervals of one week. The dose used in the army has been as follows:

First dose	500,000,000 bacilli
Second dose	1,000,000,000 bacilli
Third dose	1,000,000,000 bacilli

Although it is not an absolute protection against typhoid, yet its practical value was amply demonstrated in the World War.

Tuberculosis.—Most individuals who live in crowded communities become infected with tuberculosis during the first fifteen or twenty years of their lives; consequently, the adult possesses considerable immunity to the disease. Rarely is it possible to form an opinion as to when the infection took place, and in no single instance has it ever been possible even to guess at the size of the infecting dose. Truly, this is a most haphazard vaccination, yet so effective is it that only one out of every ten infected succumbs to the disease. This has caused various students of the subject to ask: Is it not illogical to permit this

chance infection when it is those who receive the excessive doses who succumb? Is it not possible to carefully control the dose and obtain the immunity without the dangers which accompany the natural haphazard method? Some investigators have attempted to answer those questions by experiments on cattle. They have used very small and increasing doses of the living bacilli with what appears to be promising results. Also a few carefully guarded and conducted tests have been made on human beings who were living under conditions such that natural infection was inevitable. The results have been very encouraging. However, there has as yet been no one to seriously advocate universal vaccination with living bacilli. The nearest approach to it is the work being conducted by Calmette and Guérin in France. They use an organism of the bovine type which has been cultivated for long periods in the presence of bile. They claim that the bacteria have been modified morphologically and in virulence so that a medium sized young ox will tolerate 100 mg. of such a culture, whereas a control animal, injected with 3 mg. of the same strain grown upon ordinary potato medium, succumbs in about a month to acute miliary tuberculosis. The animal receiving the culture from the bile-containing medium suffers only from a short febrile illness, which disappears in fifteen to twenty days, and is not associated with the development of any tubercles. It results, nevertheless, in an abundant production of antibodies. Animals treated with this vaccine develop a high degree of immunity toward intravenous injections of virulent bacilli.

The vaccine is known as the "B.C.G." (Bacille Calmette-Guérin). It was carefully tested on guinea-pigs, cattle and monkeys until Calmette felt sure it had completely lost its power of causing disease but retained its power of producing immunity.

In July, 1921, he began the immunization of infants. Children of parents suffering with tuberculosis were selected, who would early receive the highly virulent organisms, consequently, Calmette argued, it would not be wrong to try to protect such children by the giving of a highly attenuated culture. The organisms were administered orally during the first few days of extra-uterine life when the intestinal mucosa is readily permeable to bacteria and before the child had become infected with virulent tubercle bacilli. Since then it has been administered to many thousands of children in France and to a lesser extent elsewhere. The majority of workers who have actually tested the qualities of the B.C.G. culture testify to its safeness, and attempts which

have been made to restore its virulence have failed. Calmette maintains that it greatly protects the vaccinated child, but the more conservative feel it is too early to draw definite conclusions as to its exact value. However, if it meets the expectations and claims of its originators, it will be one of the great advances which have been made in preventive medicine, and the medical dream of a diseaseless future will have moved one notch nearer its realization.

CHAPTER XXXVIII

THE PYOGENIC COCCI

THERE is a group of parasitic bacteria constantly inhabiting the exposed surfaces, skin, and mucous membranes of man and the lower animals. They are "opportunists" lying in wait for any break which may occur in the outer defenses. Ordinarily they do not force their way through the healthy unbroken coverings of the body, but when these are damaged by mechanical, chemical, or even biological means they invade and do slight or even fatal damage, depending upon the host and the virulence of the invader. Having entered the tissues of the body they are ordinarily unable to escape in sufficient numbers to cause progressive disease of like nature in the tissues of other animals. They are locked up as it were and perish, but when conditions are made optimum for their spread from host to host, as it often was before the days of aseptic surgery, their virulence increases. Hence, they spread through the hospitals and become the great destroyer of their inmates and the terror of the surgeons. They cause inflammation followed by a purulent exudate commonly referred to as pus; hence, their name "pyogenic." Although many organisms possess this property, yet the most important members are either staphylococci or streptococci. There are different varieties or races of each of these, the type species being *Staphylococcus aureus* and *Streptococcus pyogenes*, each of which we shall now consider more in detail.

Staphylococci.—The staphylococci are quite widely distributed in nature. The parasitic forms are usually found in association with man or the lower animals. They are occasionally found in the dust of houses, and occur in hospitals if proper precautions are not exercised. They are common upon the skin, the nose, mouth, eyes, and ears of man. They are nearly always present beneath the fingernails and sometimes occur in the feces, especially of children. They are common in many skin eruptions such as "blackheads," boils, superficial and deep abscesses. They are the most common organism found in suppuration in man and the lower animals, as the following table from Karlinski shows:

		Cases
Suppuration in man	Staphylococci	144
	Streptococci	45
	Other bacteria	15
Suppuration in the lower animals	Staphylococci	45
	Streptococci	23
	Other bacteria	15
Suppuration in birds	Staphylococci	40
	Streptococci	11
	Other bacteria	20

In general, it may be stated that the lesions which they produce tend to be circumscribed rather than diffused. This is, of course, subject to exceptions, for they may at times cause a generalized condition. They do not appear to be adapted to a purely saprophytic existence and are usually associated rather closely with man and the lower animals, living a parasitic life.

Morphology.—The name "staphylococcus" has been given to this group of bacteria because of their microscopic appearance;

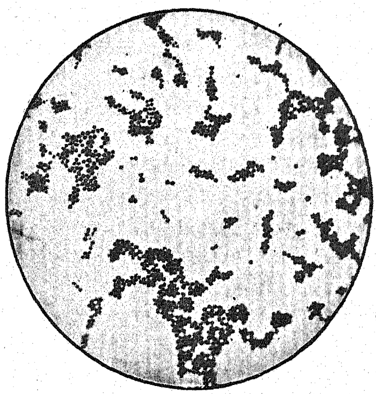


Fig. 117.—Staphylococcus aureus. Fuchsin (× 1000). (*Günther.*)

since they commonly occur in masses which have a somewhat fanciful resemblance to bunches of grapes. *Staphylococcus aureus* is the most important member of the group. The organism when occurring isolated is spherical, but when in groups has its adjacent sides somewhat flattened. It generally occurs in irregular masses but may occur singularly or in pairs and rarely in chains of four to six cocci. Its average size is from 0.7 to 0.9 micron, but occasionally either larger or smaller forms occur.

Usually the larger cocci are those about to divide. It stains well with the basic aniline dyes and is gram-positive. In fresh specimens from an acute sore many of the leukocytes will be found to contain within them one or more cocci. They are nonmotile and do not produce endospores.

Physiologic Properties.—Staphylococci grow well on all common laboratory media and at a temperature between 12° and 43° C., the optimum being about 37° C. The aureus produces a golden-yellow pigment which is especially profuse upon blood serum, or upon a starchy medium such as potatoes, or rice. However, even a good pigment-producing strain, when kept on laboratory media for some time, gradually loses its color properties. *Staphylococcus albus* produces no pigments, the growth being of a whitish color, whereas *Staphylococcus citreus* produces a bright lemon-yellow pigment in culture. The two latter are much less pathogenic than is the aureus. Some strains are almost completely nonvirulent.

The staphylococci are more resistant to adverse conditions than are the majority of nonspore-producing pathogens. They are killed only after comparatively long exposure to bright sunlight. Indirect daylight may fail to destroy their vitality even if permitted to act on these organisms for as long as two weeks. They retain their vitality during months of drying and are very resistant to low temperatures. When dry they may require a temperature of 90° C. to kill, but when moist, a ten-minute exposure of 60° C. is usually fatal. Chemical disinfectants require either longer time or stronger solutions, to render these organisms harmless, than are necessary for the majority of nonspore-forming bacteria.

Pathogenicity.—Although *Staphylococcus aureus* is pathogenic for both man and the lower animals, all the evidence points to the conclusion that man is more susceptible to it than the lower animals. These organisms ordinarily enter the tissues only after the outer protective coating has been injured in some manner, but a number of experimenters have been able to infect themselves by rubbing pure cultures on unbroken skin. They give rise to acute boils and carbuncles and, generally speaking, to acute focal inflammations which may be mild or severe, depending upon the virulence of the organisms and the tissues in which they lodge. When they locate in or on the bones they cause very serious diseases known as periostitis or osteomyelitis, depending upon the part of the bone attacked. When they reach the lungs or heart, they may under appropriate conditions

cause diseased conditions of these organs. Staphylococcic infection of the lungs sometimes occurs, and the resulting pneumonia is highly fatal. In the epidemic of influenza which swept the country in 1918–1919, this was frequently the cause of the fatal termination. This organism is often a secondary invader in pulmonary tuberculosis, diphtheria, and other severe infections. Occasionally, it may enter the blood and give rise to septicemia.

Immunity.—The immunity of man to the staphylococci is usually high and generally they enter only after the natural defenses have been broken down. Whenever the continuity of the skin and mucous membranes is destroyed, as by abrasions or weakened as in diabetes and nephritis, they invade the underlying tissue. This causes an inflammation with the congregating of the white corpuscles at the point of the attack. In most invasions the phagocytes quickly free the tissue of the invader. The immunity is probably due in a great measure to the phagocytes; but it is short-lived and may even be localized, as a patient recovering from staphylococcic infection on one part of the body may at the same time have a new infection occurring in another part.

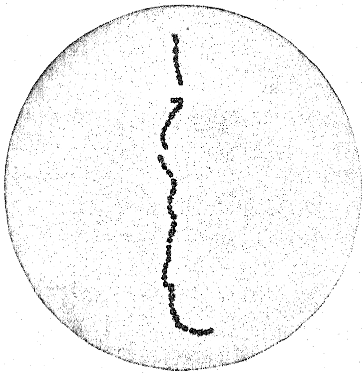
Streptococci.—Streptococci are rather widely distributed in nature. They occur under the same conditions as do the staphylococci in intimate association with man. They are to be found on every square inch of the skin and possibly even more abundantly on all exposed mucous surfaces. They are common in the mouth, nose, throat, and the intestinal tract. They also occur in soil, water, and milk. The distribution and virulence varies with the different groups. Holman lists the habitat of the five different groups which he recognizes from reactions in certain carbohydrates and upon blood as follows:

1. *Streptococcus pyogenes*—nose, throat, blood.
2. *Streptococcus anginosus*—nose, scarlet fever, endocarditis, epidemic sore throat, septicemia.
3. *Streptococcus viridans*—nose, throat, endocarditis, rheumatism.
4. *Streptococcus faecalis*—feces, milk, rheumatism, endocarditis.
5. *Streptococcus salivarius*—throat, milk, pyorrhea.

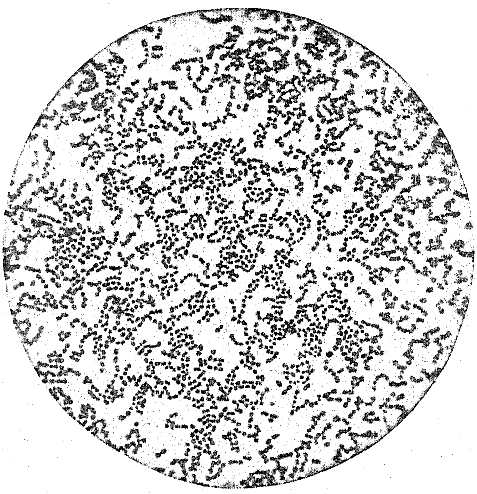
They are also grouped as hemolytic streptococci, those which dissolve red cells, and nonhemolytic streptococci, those which do not hemolyze red cells. The more virulent streptococci are of the hemolytic type.

Morphology.—Streptococci consist of spherical or oval cells

attached and forming chains. The number of cells in a chain is subject to great variations, usually running from four to twenty. Strains having eight or more in a chain are referred to as *Streptococcus longus,* shorter chains as *Streptococcus brevis.* The



A



B

Fig. 118.—Streptococci. *A* (*From Kolle and Wassermann*); *B* (*From Moser and v. Pirquet*).

longer chains are usually obtained from suppurating lesions, whereas the shorter occur more generally upon the healthy surfaces of the body. Attempts have been made to show a relationship between the pathogenicity of the organism and the length

of the chain. This has not been very successful as, at least within certain limits, it varies with the cultural media. The individual cells vary from 0.5 to 1 micron in diameter. Larger cells which are quite common in old cultures and were formerly considered to be a form of spores are called arthrospores. It is now known that they are no more resistant than the normal cells and are generally considered involution forms. All hemolytic streptococci have a great tendency to dissociate. The streptococci are non-motile without flagella and do not form endospores and stain readily with the ordinary aniline dyes.

Physiologic Properties.—The streptococci grow readily on the ordinary laboratory media, but not so well or luxuriantly as the staphylococci. They are either aerobes or facultative anaerobes. Growth occurs between 15° and 45° C., the optimum for *Streptococcus pyogenes* being 37° C. They are killed in one hour when kept at a temperature of 60° C., except when in a proteinaceous medium. They may retain their virulence for a few minutes at a temperature of 100° C. in dried sputum, and at ordinary temperature they will survive for days, or even weeks. They are quickly killed when exposed to direct sunlight but survive for some time in blood and similar media. The ordinary disinfectants are effective against them.

Considerable work has been done on the classification of the streptococci. They have been divided into groups according to their action upon carbohydrates and blood pigments. However, Rosenow and co-workers claim to have been able to transmute seventeen strains of *Streptococcus viridans* into pneumococci and twenty-one strains of hemolytic streptococci into viridans and then into pneumococci, such changes having been brought about by variations in cultural conditions and by animal passage. The cultural changes included reaction, temperature, oxygen tension, salt concentration, and growth in symbiosis. This would seem to bring into question the stability of bacterial species, but Rosenow's interpretation of the phenomenon is not generally accepted by bacteriologists.

Pathogenicity.—Man is very susceptible to invasion by the *Streptococcus pyogenes,* and once it passes the outer defences there may result local, or even generalized infection. However, their power of invading varies greatly with the different strains. Those from infected lesions, and especially those which have passed through the body of several animals, have greater invasive power for animals of the same species than those which have been growing on healthy tissue. The organism may cause local

inflammatory and suppurative processes and even generalized septicemia. Streptococci cause erysipelas and are often the cause of peritonitis, meningitis, and tonsillitis. They often play a prominent rôle in pneumonia and in tuberculosis. They are greatly feared in the cases of osteomyelitis and puerperal fever. They have given rise to numerous epidemics of septic sore throat. At one time it was believed that this disease was of bovine origin, but it is now generally conceded that the streptococcus organisms causing it come from the throats of the milker. This does not, however, exclude the possibility of the streptococci being accidentally transferred to the udder of the cow and for a time even giving rise to a disease in this animal, and secondarily in man. They often occur in the throat during measles and may cause pneumonia. They are secondary invaders in diphtheria and many gastro-intestinal diseases, and play a prominent rôle in the diseases of the lungs, particularly in the pneumonia which follows influenza. According to Gay, "Rosenow and his co-workers have shown from the standpoint of etiology that streptococci are not only present in rheumatic fever, and in endocarditis, pericarditis, and myocardial lesions, as well as the joints affected in the disease complex, but also in such apparently diverse affections as appendicitis, ulcer of the stomach and duodenum, cholecystitis, erythema nodosum, herpes zoster, mumps, myositis, iritis, iridocyclitis. Each strain of *Streptococcus viridans*, isolated from one of these conditions, will on injection intravenously into rabbits or dogs tend to localize in the same tissue and produce the same lesion in a relatively large percentage of animals as compared with the percentage of such lesions in animals inoculated with nonspecific strains of streptococcus."

Importance.—Before the days of aseptic surgery, infection, caused by the pyogenic bacteria, ran riot in hospitals. Even minor operations were usually followed by suppuration, and in major operations pus formation was so profuse that the life of the patient was always endangered. Most wounds of the abdomen were fatal, and a prudent surgeon shrank from major operations on either the head or the abdomen. We now know that the trouble came from the carrying into the tissue pyogenic bacteria, for once they gain entrance, fever, abscesses, blood poisoning, gangrene, erysipelas, one or all, may start up into ominous and fatal activity. Puerperal or "childbed fever," which is due to streptococci, used to kill one half, two thirds, or even three fourths of the women in the maternity wards. According to Williams: "In 1866 Lefort showed that in 888,312 obstetric

cases in the hospitals of France up to 1864, 30,394 women had died of puerperal fever; that is to say, 3.5 per cent, or about every twenty-seventh mother. From 1860 to 1864 the maternity mortality of Paris had risen nearly four-fold, to 12.4 per cent. In December, 1864, it rose to 57 per cent; that is to say, more than one-half of the women who bore children in that hospital in that month died of childbed fever." Similar conditions existed in other civilized countries. Hence, it is evident that an enormous toll of human life was being taken by these microbes. With the development of aseptic surgery these losses have been checked, but there are still numerous mild and many severe afflictions which are definitely known to be caused by the pyogenic bacteria. Furthermore, if we accept the theory of focal infection and its effect upon the human body, we shall have to conclude that the pyogenic cocci are still the greatest destroyers known to man.

Briefly stated, the theory of focal infection and its relation to human ills is as follows: Focal infection is a circumscribed area of tissue infected with disease-producing micro-organisms. This may be located in any part of the body, but the most common foci of infection are the teeth, tonsils, sinuses, gastro-intestinal, and genito-urinary tract. Having gained a foothold in the tissues the micro-organisms multiply, elaborate their poisons, and find their way into the lymph and blood streams. These distribute them to all parts of the body, and wherever they find suitable conditions they locate and cause disease. Hence, the vicious cycle for sore throat is: Tonsillitis, rheumatism, heart disease, and finally death. That is, according to the doctrine of focal infection, an infected tooth or a diseased tonsil is like an octopus, capable of stretching out its tentacles to such remote organs as the gallbladder, kidneys, heart, and all the vital organs of the body. Therefore, when bacteria invade and multiply in certain tissues the infection becomes a potential menace to all other tissues of the body.

Some of the diseases enumerated by Billings which may result from focal infection are:

1. Various forms of rheumatism: Rheumatic fever (with various types of heart involvement) and rheumatism of the joints, muscles, and other tissues.

2. Acute chorea, commonly called "St. Vitus' Dance," characterized by abnormal muscular movements, chiefly by twitchings, as well as nervous and mental disturbances.

3. Malignant or ulcerative endocarditis, a disease of the lining of the heart, having a high mortality.
4. Nephritis, acute or chronic kidney infections with abnormal urines.
5. Acute appendicitis.
6. Cholecystitis, acute or chronic infections of the gallbladder.
7. Gastric and duodenal ulcer, acute or chronic stomach sores.
8. Acute or chronic pancreatitis, an affliction of the pancreatic gland.
9. Erythema nodosum, a skin disturbance frequently associated with rheumatism.
10. Herpes, "shingles," or skin eruptions caused by nerve involvement.
11. Spinal myelitis, an affliction of the spinal cord influencing gait and causing dizziness, or unconsciousness.
12. Acute osteomyelitis, an affliction of the bone marrow usually occurring in the extremities.
13. Thyroiditis, swelling and tenderness of the thyroid gland.
14. Iridocyclitis, an affliction of the iris of the eye.

The evidence in favor of this theory is experimental laboratory work and the accumulated clinical experience of many medical men extending over a period of years. For example, Rosenow, in the different types of rheumatic fever, has always found at the proper stage of the disease in the joint exudate, joint capsule, circulating blood, tonsils or alveolar abscess, or other foci, certain strains of streptococci. These, when injected into rabbits, under appropriate conditions, produce the typical disease. Likewise, streptococci obtained from individuals with kidney disease, if given to suitable animals, cause infections of the kidneys. Even bacteria obtained from an infected appendix if given other animals cause a diseased appendix such as that from which the bacteria were isolated. This same method has been applied to the other diseases with similar results. These findings have been confirmed by some, whereas other workers using the same technic have obtained diametrically opposite results.

The bacteriological phase of focal infection is thus summarized by Kopeloff in "Why Infection":

"First: Certain bacteria have a special affinity for certain bodily tissues. Secondly: The members of the streptococcus-pneumococcus group are closely related and transmutable. Thirdly: Streptococci having a special affinity for certain tissues may be isolated from other bodily organs, the teeth and tonsils being especially favorable sites, from which further specific infec-

tions may be set up in remote organs. In the last analysis, therefore, the doctrine of focal infection depends upon certain important bacteriological findings. How valid are they?"

Granting that focal infection plays an important part in the afflictions of man, its significance becomes obvious if we consider a few of the diseases in which it is an important factor.

Effects of Focal Infection.—Heart disease is now first on the list of the causes of death. It is also first from the amount of

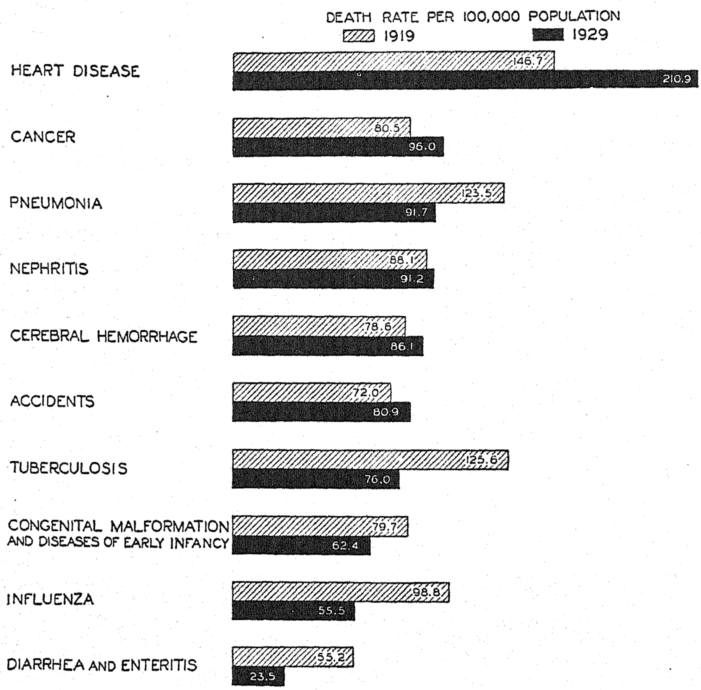


Fig. 119.—Comparison of death rates. Ten leading causes of death United States Registration Area—1919 and 1929. (*From Whitney, Facts and Figures about Tuberculosis.*)

damage it does in producing disability and invalidism, for nearly two hundred thousand persons die from it annually in the United States. If the present situation continues unchecked, one in every five of the population now living will eventually succumb to this disease. Although the greater number of the deaths are among those past the prime of life, yet many young from all walks of life are numbered among its victims.

It not only kills but it incapacitates. It is estimated that for every death from heart disease that occurs annually there are probably ten persons living impaired and deficient lives because of the breaking down of the heart function. Hence, there are approximately two million people in the United States, or about 2 per cent of the population, suffering from some form of heart trouble. Dublin estimates that fully 25 per cent of all heart disease comes from acute rheumatic fever and that the most common form of heart trouble is associated with hardening of the arteries. Forty per cent of all cases are of this type. Both rheumatism and hardening of the arteries are quite generally conceded to be caused by some form of focal infection. Hence, it is conservative to charge fully one half of the death and invalidism which result from heart trouble in the ultimate analysis, to the pyogenic bacteria.

In addition to this, approximately one hundred thousand persons die annually in the United States from kidney disorders, fifteen thousand from appendicitis, six thousand from puerperal septicemia, and nearly three thousand from erysipelas. Some of these afflictions are due entirely to the pyogenic cocci, others only in part, but it is evident from the list that these organisms outrank such plagues as pneumonia and tuberculosis as destroyers of man.

Prevention.—The pyogenic cocci are "opportunists" and usually enter the body of their host only after the natural defenses are reduced. It is evident that the greatest security against their attack comes from maintaining the body in its highest state of efficiency. Injuries to the skin and mucous membranes should receive proper attention. Overheating, exposure to cold, fatigue, and excesses in general which may render the individual more susceptible to respiratory diseases should be carefully guarded against. Measles, influenza, and other communicable diseases render the body more susceptible to invasion by pyogenic cocci. The prevention of these diseases not only reduces the mortality from this cause but also prevents many sequels due to these secondary invaders. Common colds are often looked upon as of small importance, but probably in a majority of cases they are the direct cause of the infected sinus and throat which later become permanent foci of infection contributing to the many diseases considered above. A common cold is not only an inconvenience lasting a day or so, but it is a potential danger which may in time be the cause of permanent injury, invalidism, or even death. Hence, the avoidance of colds

is a great preventive measure and the keynote of avoidance rests on remembering that they are communicable and are conveyed the same as other communicable diseases.

Recent discoveries in nutrition offer excellent suggestive material. Diets low in vitamins A and B apparently render animals more susceptible to sinus infection, and it is quite well established that proper diets containing sufficient calcium salts and an appropriate amount of vitamins D and C, if given early in the life of the individual, will protect against tooth troubles and thus prevent much focal infection.

The advice of Billings is sound: "As far as possible, as individuals and collectively, physicians should exert an influence to promote cleanliness of mind and body and thus lessen the incidence of focal and systemic infection. The encouragement of personal cleanliness and especially the care of the skin and its appendages, and the mouth and throat, should be a duty of the family physician. The necessity of cleansing the mouth, teeth and throat of all particles of food after eating should be taught as a prevention of focal infection, decay of teeth and general disease."

The findings concerning focal infection should add a new incentive for the care of the teeth, the prevention of colds, sore throat, catarrh, the proper care of ear and nose infections, and especially the maintenance of proper hygiene and nutrition. It should always be borne in mind that the best time to be concerned about focal infection is before it occurs, not after.

CHAPTER XXXIX

PNEUMONIA

The part of the body attacked and the nature of the combat varies with the micro-organisms. Each germ has its favorite point of attack: Diphtheria and scarlet fever attack the throat; dysentery, typhoid, and cholera, the bowels; bronchitis, influenza, tuberculosis, and pneumonia, the lungs. The deadliest of all diseases are those which attack the lungs. Headed by pneumonia and followed by tuberculosis, influenza, and bronchitis, these diseases account for from one fourth to one third of the annual death rate.

There is nothing slow and insidious about pneumonia. One moment its victim is well and strong; the next there is a stabbing pain in the chest, a teeth-chattering chill, followed by a high fever, and a flushed face. There is a cough, a rusty, blood-stained expectoration, and the victim is in the midst of a life and death struggle. The combat is short, sharp, and decisive. If the patient is strong enough to get the best of his assailant and is among the fortunate three fourths, the assailant suddenly gives up and departs just as abruptly as he came. The triumph over the arch enemy is just as wonderful as the bursting forth of the sun after a hurricane, but like the hurricane it leaves the earth strewn with its victims.

Historical.—Pneumonia was known and recognized as a highly fatal disease of the lungs by the father of medicine, Hippocrates. He associated it with conditions of the weather and taught that cold northeasterly winds brought on disorders of the breast, sides, and lungs. Apparently the contagious nature of the disease was not recognized until the beginning of the sixteenth century. Since then numerous epidemics of the disease have been noted. For centuries it has been observed that it is more prevalent and more highly fatal under crowded conditions, such as occur in prisons, mining camps, and armies. The pneumococcus was first seen by Sternberg, late Surgeon General of U. S. Army in 1880. He found it in his own saliva and learned that when it is injected into rabbits their blood soon teemed with the micro-organism. The same year Pasteur independently saw and de-

scribed the germ, but it was not until 1884 that Fränkel made a minute study of the organism and suggested it as the cause of pneumonia. Since that time it has been shown that pneumonia may be caused by a number of bacteria which may find their way into the lung tissue, but the principal offender is the pneumococcus.

Extent of the Disease.—As the cause of death, pneumonia is first on the list of infectious diseases and is exceeded only by heart trouble and, possibly, cancer in the number of deaths it causes. During 1928 it caused over 112,000 deaths in the United States alone. It averaged a victim every five minutes night and day throughout the year. It caused more than three times the number of deaths caused by typhoid, malaria, smallpox, measles, scarlet fever, whooping cough, and diphtheria com-

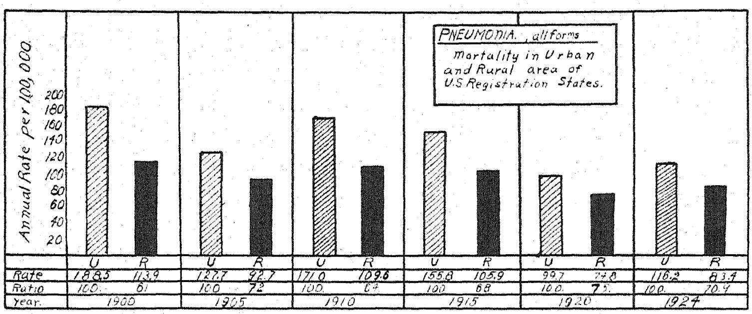


Fig. 120.—Pneumonia in city and rural districts.

bined. However, the most depressing point of all is that apparently pneumonia is on the increase. This is due to a number of factors: (1) Crowding, which favors spread; (2) the devitalizing effect of present day life; (3) the greater number of individuals who live to the age when susceptibility to the disease increases. The death rate from pneumonia per 100,000 in 1910 for individuals over sixty years of age in the United States was 734. (4) Modern methods have made the diagnosis of the disease more accurate; hence, more cases are recognized than was formerly the case. This would cause an apparent increase.

Pneumonia is considerably more prevalent in the city than in the country, as may be seen from Fig. 120. Whenever great numbers of people are brought together the opportunity for disease spreading is increased. Out of the 4,000,000 men in the

army during the World War, 40,000 perished in combat, and about 47,000 died from disease. Pneumonia caused more than 50 per cent of the deaths prior to the influenza pandemic in 1918 and 93.7 per cent during the period covered by the pandemic. Excluding the influenza period from consideration, pneumonia was nine times more frequent among the men in the army than among civilians of the same age. Pneumonia attacks all ages, but it is more prevalent among two extremes—the child under six and the adult over sixty. Vaughan writes, "Under ordinary conditions the pneumonias reap their richest harvest at the two extremes of life. During the first year there is only one other disease, infantile diarrhea, which is more fatal. Among the aged and those suffering from chronic diseases pneumonia is the kindly friend who, having prepared the bed for the body worn out with the work and worry of life's short day, administers the soporific which induces the sleep of the eternal night. On the whole, the old man rowed across the Styx by pneumonia is not compelled to pay a heavy toll in pain." Hence, Osler states: "Pneumonia may well be called the friend of the aged. Taken off by it in an acute, short, not often painful illness, the old escape those 'cold gradations of decay' that make the last stage of all so distressing."

Pneumonia occurs in all climates but shows geographical differences. It is said to be more fatal in the United States than in England. According to some authorities this is due to the dry, over-heated air of our homes, offices, and work rooms. It is more prevalent in the winter and spring months. This is not directly due to the cold, but probably to other predisposing factors such as influenza and common colds, and especially to the overcrowding which occurs at these seasons.

Types of Pneumonia.—Pneumonia occurs in two recognized forms: (1) The lobar, fibrinous, or croupous pneumonia; and (2) lobular, or bronchopneumonia. In lobar pneumonia one or more lobes of the lungs are involved in an inflammatory process. In this type there is practically always one group of bacteria found in the lungs and frequently in the blood, the pneumococci. It is a disease with well-defined, uniform onset, usually with an initial chill, rapid elevation of temperature, pain in the side, cough, and bloody expectoration. In favorable cases it runs a fever of about seven days and terminates abruptly. Bronchopneumonia is caused by a variety of micro-organisms. The pneumococcus is the most common single cause, but many others which are common inhabitants of the mouth may give rise to

the disease. In this type the bronchi, or small air passages, and even the smallest ramifications in the lungs together with the adjacent or terminal air vesicles, and the neighboring lung are the site of the inflammation. It does not present the clear-cut symptoms of the lobar type but occurs in various modifications, depending upon the extent of the lung involvement. This is the type that is most common in children.

Causes.—In pneumonia one can distinguish the immediate cause, the germs, and the predisposing or banal causes. It is not well understood how the predisposing causes act, but they in some way lower vitality so that the germ can enter. The relationship of age, season, city life, and geographical distribution has already been considered.

It has long been recognized that chronic drinkers are prone to contract pneumonia, and the outlook for such individuals when they do contract the disease is grave. Hunger, fatigue, wet, and cold all appear to be predisposing causes. It is generally recognized that a patient suffering with any of the debilitating diseases such as diabetes, nephritis, typhoid, and measles is more likely to contract pneumonia than is a healthy individual. But, after all is said, it must be remembered that the direct cause is a specific micro-organism which, in the case of lobar pneumonia, is nearly always the pneumococcus, as may be seen from the following results obtained at the Rockefeller Institute and extending over several years:

MICROÖRGANISM ASSOCIATED WITH LOBAR PNEUMONIA

	No. of cases
Pneumococcus	754
Streptococcus pyogenes	7
Influenza	6
Mixed infection	6
Staphylococcus aureus	3
Friedländer's bacillus	3
Streptococcus mucosus	1

The Bacterial Cause of Pneumonia.—The pneumococcus is usually the immediate cause of lobar pneumonia and is the most common single cause of bronchopneumonia. It is a small micrococcus known by a number of names—Diplococcus pneumoniae, Micrococcus lanceolatus, Fränkel's pneumococcus, or more briefly, the pneumococcus. It occurs most commonly in pairs, each organism being roughly oval with one extremity oval and

the other pointed. This gives to it a resemblance of the flame of a candle, or the head of a lance. Usually the rounded ends of the two cocci are adjacent. At times, especially when grown on artificial media, it may occur as chains. Its longest diameter is about 1 μ.

A well-defined capsule usually surrounds the pneumococcus. This is especially true when the organism is taken direct from body exudates, or when grown in body fluids such as milk and blood. Those cases which have high mortality usually are caused

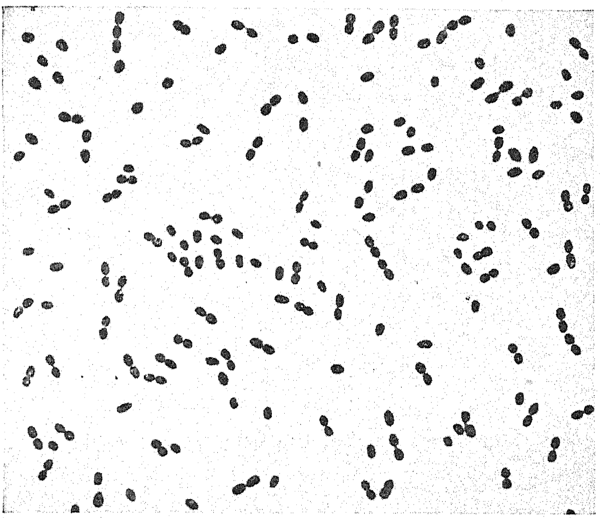


Fig. 121.—Pneumococcus Type I. (*From Ford, A Text-book of Bacteriology.*)

by definitely capsulated organisms. These observations have led to the conclusion that the capsule has a protective influence and shields the organisms against the body tissues. The organism does not produce spores, is nonmotile, and stains readily with the aniline dyes.

Types of Pneumococci.—Biological tests have shown that different races of the pneumococci occur. It is found that if a horse is repeatedly inoculated with definite strains of the pneumococcus the animal's resistance is increased, and eventually the serum of its blood when injected into white mice protects them against otherwise fatal doses of this particular strain of the pneumococcus but will not protect against other strains.

Furthermore, it is also found that such a serum has the power of agglutinating the strain which has been used but no others. By such tests it has been found that the pneumococci may be grouped into types which are indicated by the Roman numerals I, II, III, IV. The relative frequency of the occurrence of the various types together with the mortality of each is given below:

Type of pneumococci	Relative incidence	Mortality percentage
I	33.3	25–30
II	33.5	25–30
III	13.0	50–60
IV	20.3	Usually a low mortality

The importance of this grouping rests in the fact that for some types there is a quite effective serum. For instance, in Type I the mortality is normally from 25–30 per cent of the individuals contracting the disease. However, when the serum is given the mortality is reduced to from 6–8 per cent. The bacteria comprising Types III and IV are morphologically and culturally different from each other as well as from Types I and II, but Types I and II are indistinguishable from each other except by the agglutination reaction. Hence, when a patient is suffering from pneumonia the organisms are typed to determine whether the serum should be given. The typing is made as follows: A bit of the sputum is well washed, emulsified in a salt solution, and injected into the peritoneal cavity of a mouse. In from five to eight hours the peritoneum contains a strong and fairly pure growth of pneumococci. This is washed out with salt solution and centrifuged. The bacteria are then suspended in a salt solution and tested against serum from horses which have been treated with known types. At other times the culture for the agglutination test is obtained by inoculating the washed sputum into broth containing glucose and defibrinated rabbit blood. After from five to six hours the resulting growth is tested with the various type-agglutinating serums. Good results have followed the use of the serum in Type I, encouraging results with Type II, and results of doubtful value in Type III. No serum has been prepared for Type IV, as it is composed of a great variety of organisms.

Distribution and Sources of Infection.—Pneumococci are apparently normal inhabitants of the human mouth and throat,

for bacteriological examinations have shown them to be present in the mouth and throat of over one half of the individuals examined. This fact at first led to the belief that the healthy human carrier was the source of pneumonia. After it became possible to type the pneumococci, it was found, however, that most pneumococci found in the mouth of healthy individuals belong to Type IV. Types I and II seldom, if ever, are found in the mouth and throat except in individuals suffering with the disease, recently convalescent from it, or those who have been in contact with active cases of the disease. Hence, in so far as Types I and II are concerned the sources of infection are the discharges of the nose and throat of the ill, of the recently convalescent, and of those who have been in contact with active cases. There is some evidence that the organisms may survive for a short time in dust; hence, rooms recently occupied by a pneumonia patient may be a source of danger.

Most of the pneumococci found in the mouth of the healthy carriers belong to Type IV, with possibly a few of Type III. The healthy carrier is of importance in these types of pneumonia, but even here the danger is greatly mitigated by the fact that Type IV has a very low mortality. Furthermore, those carried by the healthy person are likely to be of low virulence and invade only after the resistance is broken down by disease, cold, fatigue, hunger, and so on. Hence, it can be concluded that pneumonia is spread by direct contact with infected persons or articles freshly soiled with the discharges from the nose or throat of infected persons, and possibly to a very limited extent by dust of rooms occupied by infected persons.

Properties of the Pneumococci.—Some pneumococci grow on the ordinary culture media but never luxuriantly. In fact, they usually die in less than a week on such media if kept at body temperature. They usually retain their vitality considerably longer in the ice chest, especially if light and air are excluded. They must be transferred frequently in order to keep them alive, and it is customary at intervals to pass them through the body of a mouse in order to maintain their virulence. Their temperature range is narrow, growth taking place as a rule between 25° and 42° C. They are killed by heating to 52° C. for ten minutes and are quickly destroyed by the ordinary disinfectants. They may live and maintain their virulence for months in dried sputum and withstand low temperatures very well. All pneumococci are soluble in animal bile. This is used largely to differentiate them from the streptococci.

Pathogenicity.—The susceptibility of different species of animals to the pneumococci varies widely. Mice and rabbits are very susceptible, whereas chickens and pigeons are immune. All clinical evidence points to the conclusion that man's natural immunity to the pneumococci is comparatively high, probably midway between the two groups cited. A factor of the greatest importance is the variation in the virulence. Pneumococci may be rendered extremely virulent for mice by animal passage, but on cultivation on artificial media they lose this property just as rapidly. As a general rule, Types I and II tend to maintain their virulence better than do those of type III. It is probable that there is a wide variation in the immunity of human beings toward the various strains of pneumococci. Yet it is remarkable how uniform the susceptibility is among experimental animals. For instance, it is found that if the minimum lethal dose of a number of cultures for a number of mice be determined, it is found that practically all mice can be infected with this dose.

Immunity.—Second attacks are more common in pneumonia than in any of the other acute diseases. This has led some to conclude that an attack of pneumonia produces no lasting immunity. One attack often appears to predispose to subsequent attacks. However, it is evident that there must be a short immunity; otherwise, recovery would be less frequent. Vaughan, in commenting on the greater prevalence of pneumonia among the soldiers from the rural districts as compared with those of the city, recognizes an acquired immunity, when he states: "The figures which we present convince us that the greater susceptibility to pneumonia of the soldier from the rural district as compared with his comrade from the urban home is due to the fact that the former has not acquired the degree of resistance to this disease possessed by the latter. Findings such as these, together with many others, have caused some authorities to conclude that there is an acquired immunity in pneumonia, but that it is specific and that second, third, and subsequent attacks of pneumonia come from different races or types. The clinical evidence so far obtained, therefore, indicates that following an attack of pneumonia due to pneumococci of Types I or II there may be present a considerable grade of immunity against infection with the respective types of pneumococci. Following pneumonia due to pneumococci of Type III, on the other hand, the evidence is inconclusive that the attack is followed by any great or prolonged immunity against these organisms. So far as pneumonia due to pneumococci of Type IV is concerned, there is no clinical

evidence that an attack of pneumonia due to an organism of this group is followed by an immunity against other strains of this group or against pneumococci of the other types."

How to Prevent Pneumonia.—The first and most important thing in the prevention of pneumonia is the avoidance of the germ. Pneumonia is a communicable disease and is spread directly from individual to individual. Unnecessary contact with those ill with, or recently convalescent from, pneumonia should be avoided. If one's duties are in the sick room care should be taken to cleanse the hands thoroughly, especially before eating. The sputum of the patient should be collected and burned. All utensils which come in contact with the patient should be disinfected and kept solely for his use. Food coming from the sick room should be burned. Bedding and personal clothing of the patient should be boiled.

The pneumococci multiply only in the human individual and are spread by direct contact. Special precautions should, thus be taken to prevent the spread of the secretions of the nose and throat. Foreign objects, including the fingers, should be kept out of the nose and mouth. Promiscuous spitting, careless sneezing and coughing should be avoided, as they tend to spread pneumonia as well as colds and influenza. There are carriers of the disease, whose immunity is high enough to keep in check the organism, but there is no assurance that this will be the case when this same organism is transferred to the mouth of a second individual.

One should avoid crowds in so far as practicable. This is especially true during epidemics, for normal individuals are carrying low virulent pneumococci, but during epidemics the virulent strain is often, also, present. The evidence is conclusive that overcrowding is a very important factor in the spread of the disease. It is especially important that crowds be avoided when the respiratory passage of a person is inflamed by a cold. A catarrhal condition predisposes to pneumonia, and it is safe to say that in every crowd there is a carrier of at least some type of the germ.

Undue exposure to wet and cold should be avoided, and the air of the living, working, and sleeping rooms should be clean, cool, and of the proper humidity. Excessive heat is injurious. Anything that causes a congestion of the mucous membranes of the lungs gives the pneumococci a chance to develop.

Diseases such as typhoid, measles, and diabetes render one more susceptible to pneumonia, consequently, these diseases

should be prevented, for they directly endanger life and also are predisposing to pneumonia. Most of the deaths which occur in measles are due directly to pneumonia.

The general health should be guarded. One should receive proper food, sufficient sleep, and a reasonable amount of physical exercise taken regularly and systematically; worry, overwork, overeating, unnecessary exposure, and undue fatigue should be avoided. It is at least possible that more persons are succumbing each year to pneumonia, because life is becoming increasingly more difficult. "We hurry too much, we worry too much, we play too little. Our lives are top-heavy, and the equilibrium is unstable. This is obvious in theory but we do not act upon it. Most of us get our fresh air through a distant, narrowly-opened window, our recreation at the movies, our physical exercise in walking two blocks to the cars."

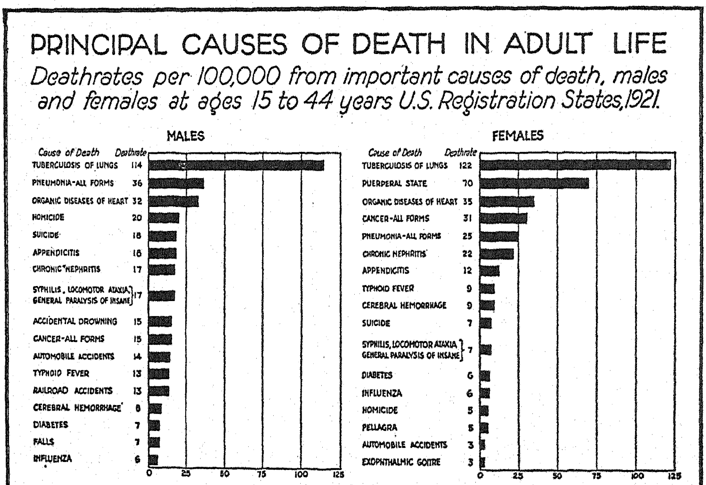
However, it would be wrong for one to conclude that physical fitness alone protects against pneumonia, for, "among the robust and vigorous of all ages pneumonia is the cowardly assassin, who, watching the every ebb in the flow of life's forces, strikes at a vital part at the most opportune moment. It has long been a matter of observation that epidemics are most fatal among the most virile. An old writer, discussing typhus fever in England and Ireland said that death went abroad among the community, picking out the lustiest and handsomest, even as you and I should go through a flock of sheep. The Typhoid Commission in 1898 found that more than 90 per cent of the men who developed typhoid fever had no preceding intestinal disorders. In the World War in the midst of the great epidemics of influenza and pneumonia, it was a common observation that the most robust were most severely stricken and supplied the largest percentage of fatalities." This is due, in a measure, to their robust condition carrying them into the danger and keeping them about when they should be receiving hospital treatment. But this is not the only factor, for Vaughan argues further "the infectious diseases owe their lesions and symptoms to the destruction of the invading micro-organisms by secretions from the body cells. The strong and vigorous man pours out these specific secretions in such abundance that enough bacterial cells are split within a short time to set free a sufficent amount of poison to lead to a fatal result. Nature over-reaches herself in the combat. Nothing worse could happen to a man with typhoid fever than a sudden disruption and destruction of all the bacterial cells in his body. Certainly, this would be true if such disrup-

tion should occur after the invading cells had accumulated in sufficient numbers to furnish a fatal dose of the protein poison on their disruption. In epidemics the body cells of the strong and vigorous man go into action with energy, and such a man either recovers promptly, or is more likely to die than his less robust comrade whose body cells destroy less abundantly and less rapidly the invading organism. Epidemic diseases certainly do not improve the race by killing off the unfit, but like war they destroy the flowers of the race, and in so doing rob the future generations of the inheritance of certain virtues. Victory in combat with epidemic diseases is not always to the strong."

CHAPTER XL

TUBERCULOSIS

The events which burn themselves into the human mind are the tragedies, the quick and sudden changes, the catastrophes of sea and land. Survivors long remember and relate the fury of the hurricane, the sorrows of the shipwreck, the suffering from the earthquake, the want and misery from a great conflagration, and the terrible loss of life from a swift acting plague,



In early adult life, deathrates of females exceed those of males for tuberculosis of the lungs, organic diseases of heart, chronic nephritis, cancer and cerebral hemorrhage. Deaths in the puerperal state are second in importance to tuberculosis among adult females.

Fig. 122.—Principal causes of death in adult life. (U. S. 1921.) (*After Dublin.*)

or war; but the slow and relentless foes of mankind are noted, commented upon, and taken as a matter of fact. Two of man's greatest microbial destroyers are the pneumococci and tubercle bacilli. They take greater tolls of human life in peace and war, on land and sea, than the more spectacular tragedies which command our attention. They have in common a special preference

for the respiratory system, but their injury is not alone confined to the lungs. Each may invade and destroy nearly any tissue of the body. They are alike in that they invade the body of man best when general vitality has been reduced by chemical, physical, or biological means. As pointed out in the preceding chapter the pneumococci strike quickly and without previous warning. The tubercle bacilli are slow and insidious. The combat between man and the pneumococci is short, sharp, and decisive. Even when man is victorious he is weakened for the next struggle, for the resulting immunity is low and short-lived. The struggle with the tubercle bacilli is long, for the powers of the combatants are usually nicely balanced, and it often results in a draw; but when man is once triumphant, there is a long and lasting immunity. The victims of pneumonia are probably on the increase, whereas those of tuberculosis have shown a steady and consistent decline during the past half century.

History.—Tuberculosis probably claimed its victims from among the lower animals long before the advent of man. There is evidence gleaned from the unmistakable scars on ancient Egyptian mummies, inscriptions on old Babylonian tablets, and early Greek manuscripts that it worked havoc even among the early human races. The Veda of India, the Zend-Avesta of the Parsees, and the writings of Hippocrates all abound in reference to it. Hippocrates left definite evidence that he recognized it as a disease of the lungs, and one of his contemporaries considered it to be contagious. Galen taught that "it is dangerous to live with consumptives and with those whose foul breath imparts a heavy odor to the room in which they live."

Closely interwoven with these careful observations of the masters are the many fanciful theories of the other classes of people. Some taught that the disease was caused by a vicious disposition of the body juices; others, that it was due to a strange ferment; and still others, that it was caused by a sharp corroding humor falling from the brain upon the lungs. Throughout the Middle Ages, the light of science was extinguished, and it was not again until the seventeenth century that intelligent discussion of the nature of tuberculosis appeared. The term "tubercle" was employed by the anatomists of the seventeenth century to refer to the nodules of various sizes in diverse tissue, and about the middle of the century Sylvius applied the term to the nodules in the lungs of consumptives. It remained, however, for Laënnec (1781–1826) to lay the real foundation for our knowledge. He recognized the different forms of the disease,

and developed the method of auscultation by which he was able to detect the disease in the living subject.

Villemin (1827–1892) used the experimental method and proved that tuberculosis is transmissible from animal to animal. He inoculated numerous and various animals in diverse ways with sputum and other products from tuberculous men and cattle, and found that tuberculosis resulted. But even at this late date many questioned his methods and conclusions. Pidoux contended: "Experiments on animals give such and such results and you, instead of controlling them by clinical experiments and by all the accepted facts of human physiology, construct upon them a general theory of human tuberculosis and all diseases! For it you upset all the ideas already acquired. We must accept over night that phthisis falls from the clouds and that in its pathogenesis, the subject himself, habitus, hygienic surroundings, hereditary and diathesis are of no account, that all depends upon an impossible tuberculosis virus originating without doubt in a tuberculous individual who had it from some other individual, and so on to the first man, who, however, had it from nobody at all and must have created it himself out of nothing."

It remained for the glorious work of the immortal Robert Koch to reveal to the eye of man the "impossible tuberculous virus" in 1882. He not only showed that the organism is in the sputum of tuberculous patients and lesions in the various parts of the body, but he also succeeded in growing it in artificial media and demonstrating its pathogenicity. Since that day many patient workers in various lands have added to this knowledge, until today we possess several outstanding facts concerning tuberculosis. (1) It is an accepted truism that without the specific organism there can be no tuberculosis. (2) Children are born free of the disease. (3) Tuberculosis infection increases with age and when adult life is reached between 50 and 100 per cent of the population in civilized countries show signs of the infection. (4) The majority of civilized individuals get the infection, but only about one in ten develops the active disease. (5) Tuberculosis is both a preventable and a curable disease.

Prevalence and Cost.—We look back on the four years of our Civil War with its fatality of two hundred and fifty thousand killed and think what a loss it was to the nation, yet during the past three years more than this number met death in the United States from a foe which claims more victims than does war. It has been estimated that during the nineteenth century four-

teen million lost their lives on the battlefield. During the same period over thirty million died of tuberculosis. The twentieth century has been ushered in with gigantic wars, yet it is a safe guess to say that during this century more will die from the "Great White Plague" than on the battlefield.

In 1928 approximately 90,000 persons died in the United States from tuberculosis, that is, over 7 per cent of the deaths were

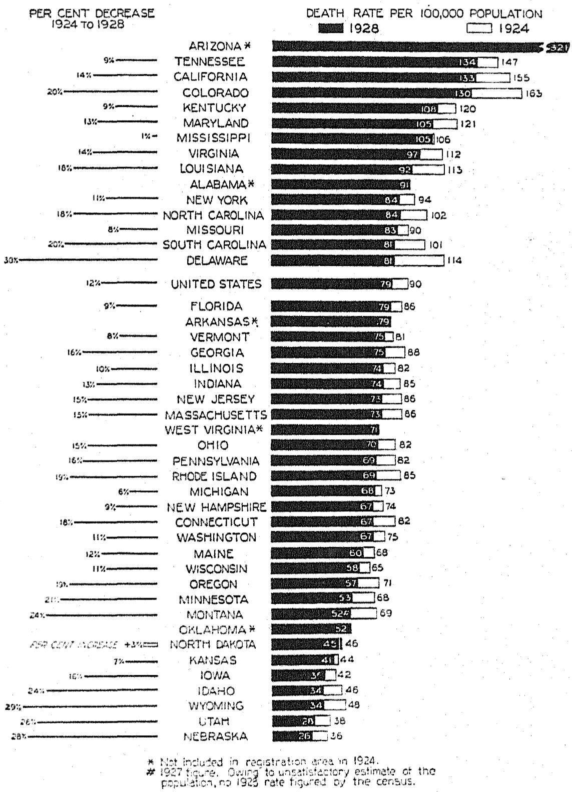


Fig. 123.—Regional incidence of tuberculosis mortality in the United States. (*From Whitney, Facts and Figures about Tuberculosis.*)

due to this disease. In some foreign countries the rate is still higher. It claims yearly over three times the victims taken by scarlet fever, measles, typhoid, diphtheria, and whooping cough combined. It is exceeded among the communicable diseases only by pneumonia. Pneumonia claims the greatest numbers at the two extremes of life, but tuberculosis takes man just when life

should mean the most to him. It has been estimated that 30 per cent of all deaths between the ages of fifteen and sixty are caused by it.

The number ill with this disease has been variously estimated at from five to twenty times the number that actually die of it. It is probably conservative to place the morbidity rate at ten times the death rate; this would mean that there are approximately 1,000,000 cases in the country, or about one per cent of the population ill with the disease. The number infected is much higher. Over 90 per cent of city dwellers show signs of

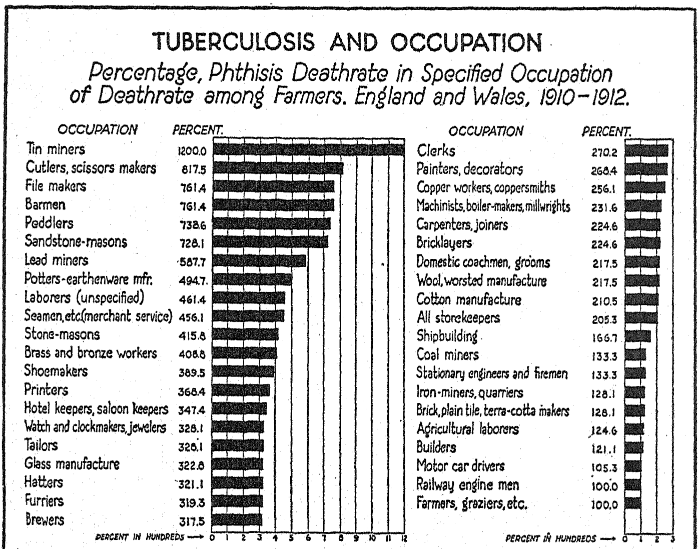


Fig. 124.—Tuberculosis and occupation. (*After Dublin.*)

tuberculosis in their bodies after death; 60 per cent show signs in the lungs. The tuberculin test which is used in living patients is so delicate that it not only reveals active and serious tuberculosis, but also the presence of minute and even practically healed small, diseased areas, in any part of the body. This test shows 90 per cent of all twelve-year-old children and 60 per cent of the young healthy adults to be tuberculous. When the whole population of city and country are considered, it is estimated that 50 to 70 per cent have been infected. Hence, while one in every fourteen deaths is due to tuberculosis, eight in

every twelve individuals become infected with the disease. In most cases, however, the infection is so slight that the patient is never conscious of it.

The mortality from tuberculosis varies widely with a number of factors. There is a greater mortality among the negro population than the white. More males than females die of the disease probably because there is a greater opportunity for infection. It is more prevalent in the city than in the country districts and in the southern than in the northern and western parts of the country. The mortality is lowest in Nebraska, Utah, and Wyoming; and highest in Arizona, Tennessee, and California. It must not, however, be concluded that the climate of these latter states is conducive to the contraction of tuberculosis, for many that die there are tuberculous patients who have flocked to these regions with the hope of regaining their health.

There is also a relationship between the tuberculosis death rate and the occupation. Statistics show miners, cutlers, scissors- and file-makers leading the list, whereas the mortality among farmers, railway engineers, and motor car drivers is low. Two factors enter: (1) The opportunity for infection which undoubtedly is greater in some occupations than in others; and (2) the conditions which are more conducive to ill health in some occupations than in others. Hazardous occupations are those in which the person is constantly inhaling sharp or irritating particles which cause small lesions in the respiratory epithelium and admit the bacilli.

During the past twenty years there has been a gradual but consistent decline in the death rate from tuberculosis. In 1900 it was 195.2 per hundred thousand of population in the United States for which reliable statistics are available. In 1910 the rate in the same geographical area had dropped to 164.7, or 15.6 per cent, in the ten-year period. In 1920 the rate in the same states was 112. This is 42.6 per cent less than the figures for 1900. In 1929 the rate reached the low figure of 76 per hundred thousand, which is considerably less than one-half of what it was in 1900.

This general decline is attributed to different factors by various workers. The first explanation which is espoused by most workers in the field of public health is that the decline in the tuberculosis death rate is due to the great improvement in the general well-being of the population. Those who hold this view say that the great mass of the population are pretty generally exposed to infection, but those who break down with the disease

are the ones who lack the immunity to localize it in the very early stages. This power of immunity is being increased by the good hygienic habits which people are learning to live.

The second explanation minimizes the importance of hygiene and stresses the constitutional factor. The people holding this opinion point to the fact that the decline in death rate antedates even the knowledge of the specific organism. They consider that it is inherent within the race. There are being produced individuals who are more resistant to the germ.

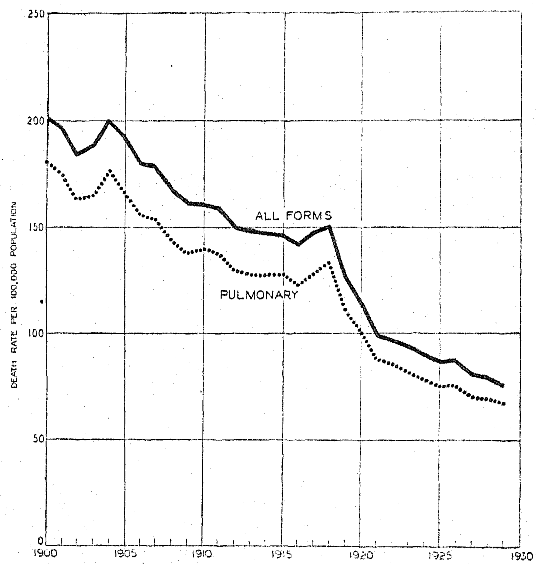


Fig. 125.—Tuberculosis death rates. All forms and pulmonary. United States Registration Area—1900 to 1929.—(*From Whitney, Facts and Figures about Tuberculosis.*)

Besides the loss in life the economic loss from the disease is appalling. It is impossible to accurately evaluate this, but it has been estimated that the yearly loss to this country from tuberculosis exceeds in value that of the wheat and corn crop, and that the loss of an annual hay, milk, butter, and wheat crop would only slightly exceed the annual loss due to tuberculosis. The disease costs approximately half a billion dollars annually in the United States—more than the army, nearly twice as much as the navy, and second only to the national debt in comparison with government expenditures.

Heredity.—The frequency with which tuberculosis occurs in some families early led to the idea that the disease was inherited, but when it was discovered that the disease is due to a germ which invades and injures it did not appear probable that it could be transmitted from parent to offspring in the germ plasm. This conclusion is borne out by extensive clinical and experimental work. There are very few cases on record in which the child is born with the disease. Consequently, it is generally accepted today that the child even of tuberculous parents is born free of the disease. In other words, tuberculosis is not inherited.

However, there has been much discussion in recent years as to whether or not a tendency toward the disease is inherited. It is quite generally conceded that some races, such as the American Indians and Negroes, succumb to tuberculosis more readily than others; but it is hard to nicely separate the hereditary tendency from the bad hygienic conditions under which the races live. Moreover, in the United States, the death rate from tuberculosis among the Jews is only one fourth of what it is among the general population, but the number of infected is probably greater than in other races. Guerm has tried to defend the idea that blondes with auburn silky hair and pale freckled skin of fine texture are especially prone to contract tuberculosis. It is probable that certain tendencies to the disease are inherited, but even this is challenged by some who point to cases in which calves of tuberculous mothers separated from their mothers at the time of birth, fed with milk from healthy cows, and shielded from infection later did not contract tuberculosis more readily than the offspring from healthy parents. Hence, while we conclude that tuberculosis is not inherited, resistance seems to be built up in a race in which the disease has long been prevalent.

Undoubtedly the main reason children of tuberculous parents are more prone to contract the disease than other children is due to the greater opportunity for infection. They are constantly exposed to infection at an age when their immunity is low. The infection may be brought about by the mother, who tastes the porridge with a spoon to assure herself that the temperature of the food is correct, who wipes the baby's eyes and mouth with her handkerchief moist with bacilli-laden sputum, or who kisses the child upon its lips. When the child becomes old enough to creep or walk it is constantly sucking its dirty fingers covered with the sputum-laden dust.

Cause of Tuberculosis.—Tuberculosis is caused by a rod-shaped organism discovered by Koch in 1882. In its absence

there can be no tuberculosis. Yet only one in ten or twelve infected with the organism die of the disease. Moreover, the work of recent years has demonstrated that the disease is curable, if it is detected in the incipient stages and the patient adheres to a carefully regulated hygienic regimen. This has led to the belief that there are predisposing or banal causes in tuberculosis. Rosenau writes: "In man the balance between immunity and susceptibility to tuberculosis is delicately adjusted; there is a very small factor of safety. The resistance to infection may be increased by attention to personal hygiene, fresh air, and good food; immunity may readily be broken down by any weakening influence; herein lies the keynote of personal prophylaxis."

Irritating fumes and sharp particles when inhaled cause lesions in the epithelium of the lungs, and open the way for the too-often present tubercle bacilli. English statistics show that if the death rate from tuberculosis among agriculturists is taken as 100, then that of tin miners is 1200, cutlers 817, file makers 761, car men 761, and stone masons 728. There are a number of complicating factors entering into a consideration of such statistics. The greater prevalence of the disease in these occupations throws the health worker in constant contact with the germ. Yet even when this is considered, it can be concluded that many dusty occupations increase the hazards of tuberculosis.

Wild animals in a state of nature are not naturally liable to the disease, but when confined in zoological gardens they quickly succumb. The cattle from the mountain ranges of the west seldom suffer from tuberculosis, and farmers, engineers, and motor drivers all show a low mortality from the disease. Emery considers exposure to a vitiated atmosphere a most potent cause of the breaking down of immunity. "It (a vitiated atmosphere) is especially important in connection with tuberculosis, and nothing is more striking than to notice its effect on the peasantry of some regions in which in spite of exposure to abundant fresh air during the daytime, and a supply of food which certainly does not fall below the physiologic minimum, and is usually more abundant, phthisis and other tuberculous diseases are rife."

The beneficial influence of fresh air is due more to the general physical effect upon the surface of the body than to its effect through the lungs. Consequently, fresh air should be obtained to as great an extent as possible in the open, and not alone by the mechanical devices which are arranged to bring it into the house.

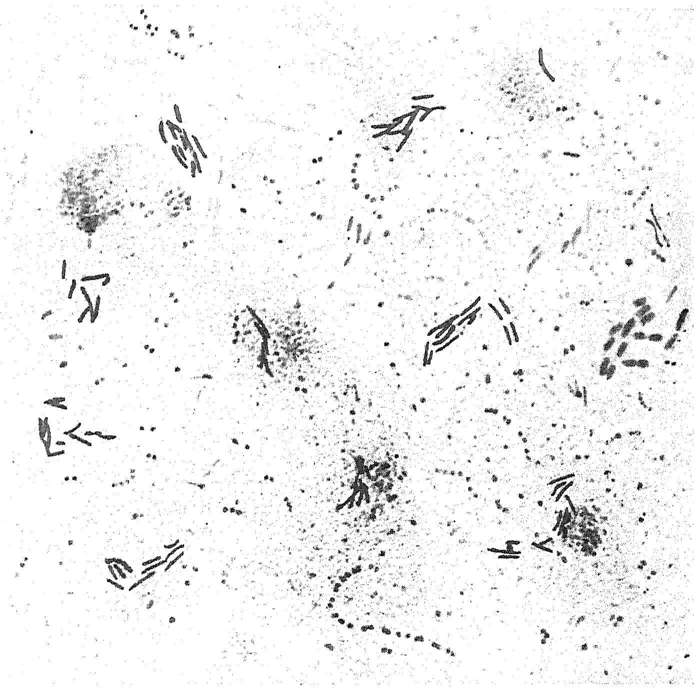


Fig. 126.—*Mycobacterium tuberculosis* from sputum. (*From Ford, A Text-book of Bacteriology.*)

Recent research indicates that the value of outdoor life is in a measure due to the action of the ultraviolet light upon the tissues. Animals as well as plants require light rays in order to properly metabolize their food. We are all children of the sun and are directly or indirectly dependent upon it for strong, healthy bodies. The harmful effects of alcohol; the wasting diseases, typhoid fever, diabetes, and whooping cough; together with fatigue, poverty, and malnutrition have long been recognized by

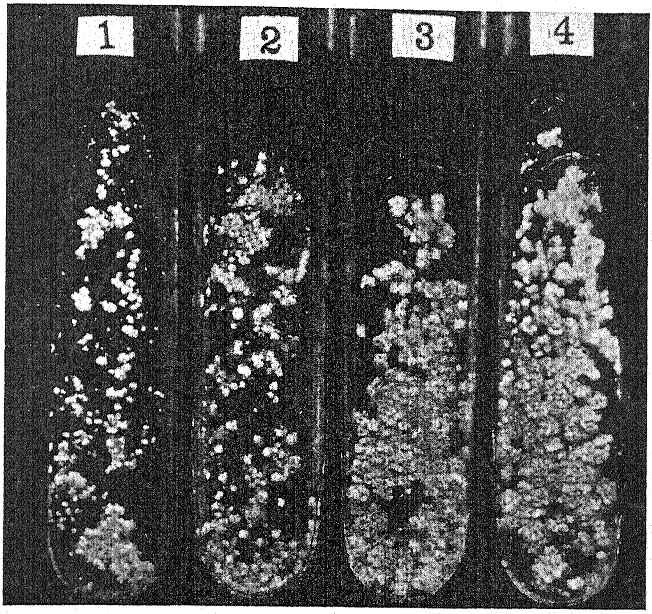


Fig. 127.—Growth of tubercle bacillus, fourteen days at 37° C. The better growth in tubes 3 and 4 is due to better moisture conditions. (*After Novy and Soule.*)

the physicians as potent factors in the breaking down of the natural immunity to tuberculosis.

However, one must not lose sight of the fact that even the strongest and most robust individuals in the prime of life succumb to tuberculosis, if they receive large virulent doses of the organism; consequently, it is essential to learn the source of the germs. They multiply only in the bodies of man and the lower animals and are found in close association with them. There are three kinds of tubercle bacilli—human, bovine, and avian.

Human Tubercle Bacillus.—This organism produces the disease primarily in man. It is a slender rod often slightly curved. It measures from 2 to 4 microns in length and 0.3 to 0.5 microns in breadth. In material taken from the body, sputum for example, it occurs singly, or in pairs usually arranged at an angle but more often lies in small heaps. In old cultures and at times from the sputum, clubbed or filamentous forms may occur. The latter are often branched. Some observers consider these as involution forms, others consider them as normal variations of the organism. The tubercle bacilli are nonmotile, possess no capsule, and do not form spores. The usual stains are without effect upon them unless heat is applied, or the stain allowed to act for a long time. When they are once stained, they are not easily decolorized. They may be treated with mineral acids or alcohol and still retain the stain. This has caused them to be referred to as "acid-fast," or "alcohol-fast." This property may probably be attributed to the large amount of waxy and fatty material which their bodies contain. They grow only on special media, blood serum, egg media, and the like. After they have been cultured some time on these special media, they will grow on glycerin agar. They grow best aerobically at a temperature of 37° C., are quite resistant to drying, and may survive in dry sputum in a dark, cool place for even months. Direct sunlight effects their destruction in a short time especially in the presence of oxygen. They are killed in the presence of moisture in fifteen to twenty minutes at a temperature of 60° C.

They are quite resistant to dry heat, for in dried sputum they are able to withstand a temperature of 100° C. for one hour. When free from proteins, they are readily destroyed by the ordinary disinfectants, but in sputum they are quite resistant because of the mucin of the saliva. Lysol has a solvent action on the mucin; hence, it is one of the better disinfectants for this purpose.

Bovine Tubercle Bacillus.—The bovine bacillus is very pathogenic for all mammals except man. It is pathogenic for man but to a less extent than is the human bacillus. A large dose of the human bacilli injected into the calf usually results in only a local lesion, whereas the bovine type gives rise to a severe form of the disease. The distinguishing test for the two is made by rabbit inoculation. If 0.01 milligram of a bovine culture is injected intravenously, or 10 milligrams subcutaneously, into a full-grown rabbit, generalized tuberculosis results in about six weeks; whereas ten to one hundred times this amount of a human

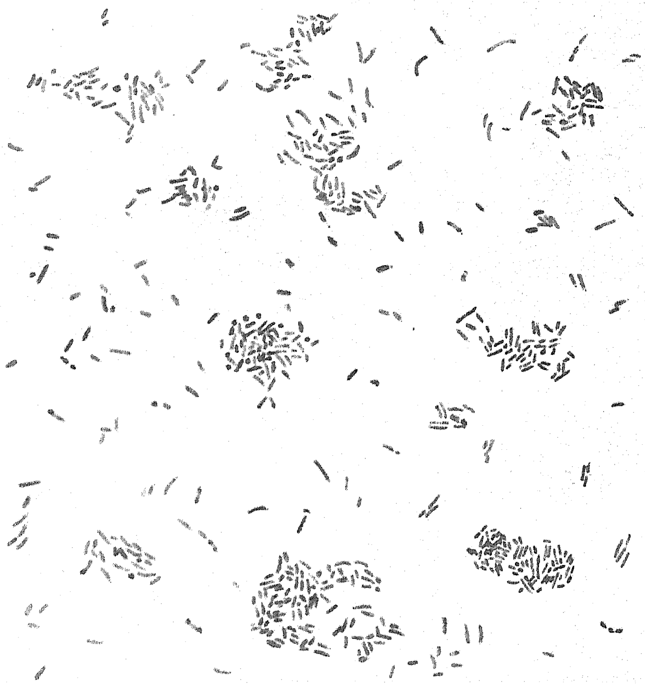


Fig. 128.—*Mycobacterium tuberculosis* bovine type, from glycerin-agar culture, fifteen weeks old. (*From Ford, A Text-book of Bacteriology.*)

strain produces at the most a slight localized tuberculosis. The bovine bacillus is shorter and thicker than the human bacillus and grows less readily upon laboratory media. The acidity curves in glycerine broth are different.

The most important phase is the relationship of the bovine bacillus to human tuberculosis. Koch in 1901 announced that there was practically no danger of man contracting tuberculosis from cattle, but his statement was immediately challenged by many bacteriologists. Since that time statistics have been collected in many parts of the world. By 1911 Park and Krumwiede had collected a total of 1224 observations, and from these they drew the following conclusions: (1) That children are often infected by the bovine bacillus, and the portal of entry is the alimentary canal; (2) that cervical adenitis and abdominal tuberculosis are the most frequent types of the infection; (3) that generalized tuberculosis due to bovine infection is less frequent; (4) that bone and joint tuberculosis is the most common of the human type; (5) that the meninges are less commonly affected by the bovine than by the human bacillus; (6) that the bovine type seldom infects adults; (7) that although cases of pulmonary tuberculosis due to the bovine type of bacillus have been reported, such cases are exceedingly rare. They estimate that about 10 per cent of all deaths caused by tuberculosis in children under five years of age are the result of bovine infection. This is an entirely unnecessary waste of human life as the proper pasteurization of all milk would prevent nearly all cases of bovine origin.

The Avian Tubercle Bacillus.—Morphologically, the avian bacillus is like the human. It grows abundantly at the surface on glycerin media and has an optimum temperature of 40° to 43° C. It readily infects chickens, whereas the human and bovine do not. Its main importance rests on the damage it does when once it gets into a flock of chickens. A few cases have been reported in which it has been found in tuberculous lesions of human individuals, but it must have a very low pathogenicity for man; otherwise, it would be more prevalent.

Tuberculin.—Robert Koch early in his study on tuberculosis learned that dead bacilli, or their extracts when injected into infected animals produce a different effect than when injected into healthy animals. This led him to the discovery of tuberculin which is a concentrated extract made from the tubercle bacilli. When injected subcutaneously a local inflammation results. There is also a general reaction characterized by headache, back-

ache, and fever. Koch advocated its use as a curative agent and believed that the reactions occurred only in tuberculous subjects. Later work showed it to have little value as a curative agent, and if not properly used might do harm. It was found that when large doses were administered both healthy and diseased animals reacted, but by nicely governing the dose it was found possible to differentiate the infected from the noninfected. Because of the extensive prevalence of infection in the human individual it has little value in human diagnostic work, but is used extensively either in its original or modified form for the

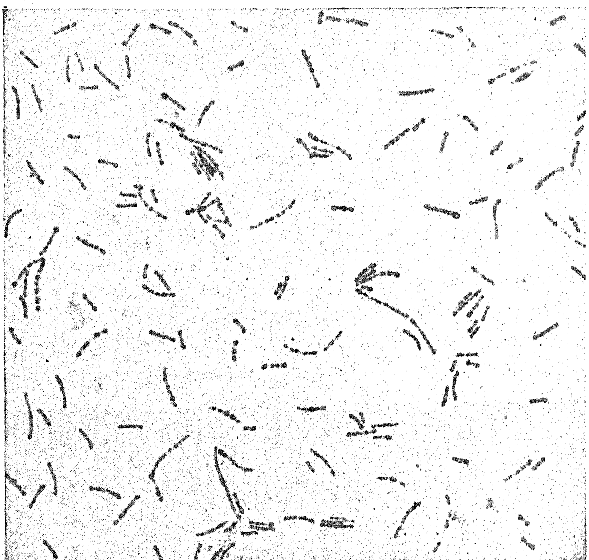


Fig. 129.—*Mycobacterium tuberculosis*, avian type, from ten weeks' culture on glycerin agar. (*From Ford, A Text-book of Bacteriology.*)

diagnosis of the disease in cattle, and if properly and systematically applied it is possible to eradicate the disease in cattle.

Calmette and Wolff-Eisner found that tuberculin dropped into the eye produced a reaction. Moro rubbed it into the skin, and finally von Pirquet in 1907 found that if a very slight abrasion was made in the skin and a drop of tuberculin placed on it a local reaction occurred in tuberculous persons without causing systemic or general reactions. At first it was believed that this test had great diagnostic value, but it was soon discovered that nearly every one with the exception of very young children,

healthy or tuberculous, reacted. This taken in connection with the findings at autopsy led to the conclusion that practically everyone becomes infected with tuberculosis at an early age, but that only a comparatively few become tuberculous.

Modes of Infection.—Since the tubercle bacilli do not grow in nature but in the body of man and the warm-blooded animals, these are the fountains of tuberculous infection. The germs leave in the excreta of the body, sputum, feces, surface sores, and, occasionally, in the urine. Inasmuch as they may survive for months in the moist or partly dried excreta, it can readily be understood how easily they can find their way into the body of human beings. The saliva of the diseased is constantly being spread on fingers, pencils, cups, toys, and a thousand and one things which are mouthed by one and then another, to say nothing of the even more direct transfer of the organisms by the kiss.

Brown and Cummings independently have shown that forks, spoons, and cups used by people with open tuberculosis carry the bacilli. It has been further shown that when such spoons are washed, as they ordinarily are washed in the home, the wash water, rinse water, and the spoons all carry tubercle bacilli. Consequently, it is evident that great care must be used in sterilizing tableware, especially forks, spoons, and glasses used in restaurants, boarding houses, and the soft drink parlors; otherwise, they may be potent factors in the spread of disease.

The sputum of the tuberculous individuals often contains millions of the tuberculous germs, and when carelessly deposited on the street or ground may reach the fingers of playing children and later their mouths. Or the bacilli may be carried on one's shoes to the floor or rugs of the dwelling rooms where small children playing about come in direct contact with them. Then there are insects which fly or crawl directly from germ-laden sputum to the food of the home or the store. The dried sputum may be brought into the air by sweeping or by air currents and in this manner infect, for the organism may live for considerable periods in dust-laden atmosphere away from the direct rays of the sun.

There is also the infection which results from the droplets forced from the mouth during coughing. Hence, it is evident that the tuberculous individual who permits the infected droplets to fly from the unprotected mouth is endangering those in his immediate vicinity. Or, if he guards the cough with the bare hand and then shakes hands with a friend, he is making the transfer even more direct.

There may also be infection from the lower animals, and where raw milk is used there is the ever-present possibility of infection, which according to the careful investigations of Park is considerable. Infection from meat and milk can be entirely prevented by pasteurizing the milk and by inspecting and proper cooking of the meat.

Channels of Infection.—It is possible for tubercle bacilli to enter the body through either the respiratory or digestive tracts. Occasionally, the germ enters through abrasions, and there are a very few cases on record where they have been transmitted from mother to fetus through the placenta. In the early history of the knowledge of tuberculosis, it was concluded that infection entered mainly through the respiratory tract. This opinion was held primarily for the following reasons: (1) Because the sputum of the diseased is often extremely rich in tubercle bacilli; consequently, on drying it may be ground and carried into the air. (2) Because the organism usually localizes in the lungs; therefore, tubercle bacilli carried by dust, together with infected droplets direct from the mouth of the ill, offered a plausible explanation for infection. It was, however, learned that ingested bacilli may localize in the lungs, and the point of the lungs in which they locate is similar to that occurring in natural infection, moreover, smaller numbers infect when ingested than when inhaled. These findings directed attention to the alimentary tract as the portal of entry. Ingestion is the sole method of conveying bovine tuberculosis to man. It has been repeatedly shown that tuberculosis can be conveyed to animals by either method, and there is abundant evidence pointing to the conclusion that the same is true of the human individual. Consequently, all who have made a careful study of the subject are agreed that the disease does result from ingestion and inhalation of the germ. But which is more important? Here even careful students of the subject part company. We cannot say.

Influence of the Bacilli on the Body.—Having entered the body of the host the effect varies greatly, depending upon the number, their virulence, and the resistance of the host. Probably in the majority of cases where the number of bacilli entering are small, they are quickly destroyed and the host develops the power of handling even larger doses, or they may lodge in some tissue and cause increased growth of epithelial and interstitial cells which soon form a globular mass which walls them off from the rest of the body. Thus result minute tubercles.

Sometimes the body succeeds in killing the bacilli. At other

times it only keeps the microbes from growing. They remain alive and latent but ready to start their campaign of conquest anew, if the effectiveness of the barrier is reduced by infection, malnutrition, overwork, fatigue, cold, and poor hygiene.

Occasionally, these tuberculous processes become chronic, the tubercle bacilli continue to grow, the infected area becomes larger and larger, and more of the organ in which they are located is destroyed. Symptoms become more pronounced because of the impaired organ, and the poisons elaborated and distributed throughout the body by the blood. In rare instances the growth may be in the walls of a venule or arteriole that eventually ruptures directly into the circulation. Once in the blood stream the bacilli are carried to various parts of the body where they may locate and give rise to new tubercles. This condition is known as miliary tuberculosis.

Whenever the tubercle bacilli find conditions within the host favorable for their nourishment and growth, the tissue adjacent to the bacilli is injured; and although new cells are formed, these in time are killed, and the area of dead tissue enlarges, so that there soon results an ulcerating sore in the tissue. This process continues, the blood supply is shut off from certain tissues, nutrition stops, the cells die, and then a cavity is formed in the living tissue. The softening area continues to necrose until it reaches a bronchus in communication with the outer world. Soon the organisms are occurring in the sputum of the victim ready to infect another.

Prevention.—The hopeful aspect of the subject is that tuberculosis is a preventable disease. It is questionable whether it will ever be possible to prevent infection. Some workers even argue that this should not be attempted unless we are able to annihilate the tubercle bacilli. However, all agree that every known means should be used to prevent the breaking down of natural immunity by massive doses of the virulent organism. This implies the prevention of the freshly infected excretion of one individual reaching the body of a second. The sputum of the tuberculous person should be collected and burned. The tuberculous patient going about spitting right and left may be just as dangerous to a community as a maniac running amuck with a machine gun. It is well to remember that the great enemy of germ life is sunshine; consequently, the sun should enter all places where people congregate.

Bovine tubercle bacilli are infectious to man; consequently, they should be prevented from entering food. This can be suc-

cessfully accomplished by the systematic testing of all animals for tuberculosis and the destruction of the diseased. In the District of Columbia the extent of tuberculosis among cattle has been reduced from 18.87 per cent in 1910 to 0.63 per cent in 1919. Moreover, since the tuberculin test has come into general use there has been a consistent decrease in the number of tuberculous animals which come to the slaughter house, as may be seen from the following:

Year	Percentage	
	Cattle	Swine
1917	0.50	0.19
1918	0.37	0.17
1919	0.33	0.15

Inspection alone cannot be depended upon, but the public must be educated to eat only well-cooked meats even though they have been inspected. All milk should be pasteurized before being used. The value of this procedure is reflected by the decrease

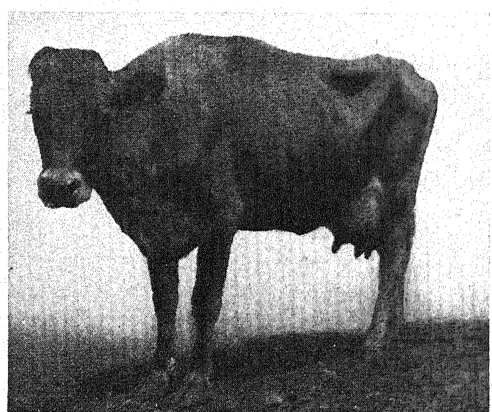


Fig. 130.—A visibly tuberculous cow. (*Courtesy of Dr. Frederick.*)

in bovine abdominal tuberculosis in New York. During the years previous to the pasteurization of its milk supply 64 per cent of the cases of abdominal and lymph node tuberculosis were of the bovine type. Today 98 per cent of the city's milk supply

is pasteurized, and only 16 per cent of the cases of lymph node and abdominal tuberculosis is due to the bovine germs. Malnutrition is the friend of the tubercle bacilli, as it reduces natural immunity. It is well to remember that much malnutrition is due to poverty, but still more is due to ignorance. It is not sufficient that the child, or the adult for that matter, fill his stomach three times a day, or that he receive the required number of calories; but, as pointed out by McCollum, the diet must contain certain dietary constituents which cannot be obtained from the muscle meats, highly refined grains, and fleshy tubers. The properly balanced diet should contain milk, a leafy vegetable, and a fresh fruit. Milk and leafy plants are especially important in furnishing calcium to the body. Considerable evidence has accumulated within recent years which shows that an abundance of calcium in the diet protects against tuberculosis. Workers who toil in an atmosphere laden with calcium-rich dust show a lower incidence to tuberculosis than laborers in other occupations.

It should constantly be borne in mind that debilitating conditions, such as insufficient exercise, overwork, worry, alcohol, and all excesses, lower natural immunity. One should sleep in well-ventilated rooms and avoid dust and other sources of irritation to the lungs. Regular systematic exercise should be taken; well-developed chests are a good insurance against tuberculosis. Colds and pneumonia should receive prompt attention. In short, a simple, regular, well-rounded life should be lived.

Cure of Tuberculosis.—Tuberculosis is one of the most curable diseases known to man, as is readily seen from the fact that from 80 to 90 per cent of the population becomes infected at one time or another during the course of their lives, and yet only 8 per cent die of tuberculosis. Each year the hopes of many sufferers are caused to burn brighter by the announcement of a sure panacea for tuberculosis, but each year sees the same individuals crushed with disappointment; consequently, today it must be stated that no drug is known which cures tuberculosis. At the present time, hopes are entertained that some gold salt may be found to effect cures, but as yet the conservative workers still counsel the use of the old tried and proved remedies—rest, fresh air, sunshine, proper food, and contentment. These are the five essentials on which humanity today must stake its fight in the prevention of infection, and on these those ill with the disease must hang their hope for recovery. When properly applied they work miracles.

The term "tuberculosis" often strikes terror to the heart of

man when applied to him or his loved ones, but the kindest act a physician can do for his patient is to notify him early if tuberculosis is present. Consequently, one of the most potent factors in its cure is the annual medical examination by a competent physician, and if tuberculosis is even suspected one should follow a carefully regulated hygienic regimen in which fresh air, rest, sunshine, proper food, and contentment are the medicines. The use of a vaccine has already been considered on pages 430 to 432.

CHAPTER XLI

DIPHTHERIA

THE Utopian dream of the bacteriologist will have been attained when he has learned to know the definite micro-organism which causes each specific disease; when he has found an exact method for diagnosing the disease, and has learned how to determine when the convalescent patient is free from the germ and no longer a danger to the community and consequently can be released from quarantine; and when he has found a preventive and a curative agent for each specific bacterial disease. All this has been accomplished in the case of diphtheria.

History.—Centuries before the Christian Era diphtheria periodically visited the human race, swiftly took its toll, and left man gazing in utter helplessness. There is good evidence that it existed in Greece long before the days of Hippocrates and it was believed by the Greeks to have come from Egypt. Galen refers to the false membrane that is found in the throat of the patient and which is often dislodged by coughing, and Aretaeus gave a fairly accurate description of the disease as we now know it. However, it was Bretonneau (1775–1862) who laid the firm foundation on which modern work has been done. He showed that diphtheria is an infectious disease and is often spread by the common drinking cup and other table utensils. He was the first to successfully relieve the patient by opening the trachea. Klebs, in 1883, first called attention to a very characteristic micro-organism which occurred in the throats of diphtheria patients. The next year Löffler obtained the germ in pure culture and grew it on laboratory media. He was unable to find it in the throats of all diphtheria patients and occasionally found it in the throat of healthy persons; consequently, he concluded that the discovered organism was not the direct cause of the disease. Four years later the mystery was solved when Roux and Yersin triumphantly proved that the Klebs-Löffler bacillus produces a soluble toxin which, when separated from the bacterial body and given to a susceptible animal, reproduces the disease with singular fidelity.

To the distinguished German, Emil Behring, a student of Koch, and to the equally distinguished Frenchman, Emile Roux, a student of Pasteur, belong the honor of discovering the life-saving antitoxin, which may be used both for the cure of the disease, and the production of a short immunity. In 1913 Schick developed a method for determining when a person has sufficient antitoxin within his body to protect him against the disease in case of exposure. Within recent years the patient, skilled, and scientific work of Park and his associates has developed a method by which a susceptible person may be given a mixture of toxin-antitoxin and thus develop a high degree of lasting immunity. Consequently, all that remains to be done in order to eradicate diphtheria is to educate the masses, so that they will take advantage of the life-saving fruits of science.

Prevalence.— Diphtheria epidemics occur in cycles. The interval between and the severity of epidemics vary widely. This has been noted for centuries, and in 1613 it was so prevalent and so deadly in Spain that in the chronicles of that time this is known as the "diphtheria year." The epidemic years in Boston were 1863–1864, 1875–1876, 1880–1881, 1889–1890, and 1894; in New York 1876–1878, 1880–1882, 1886–1888, and 1893–1894; in Chicago 1860–1865, 1869–1870, 1876–1879–1881, 1886–1887, and 1890. Various explanations have been offered to account for this phenomenon. Rosenau consideres the cycles due to a combination of three factors: (1) Man, (2) the bacillus; and (3) the environment. An epidemic may sweep through a city and practically all the susceptible individuals contract the disease. It would, then, be a number of years before there would be another susceptible group of children. There is also the likelihood that the strain of diphtheria germ may be more virulent at one time than another. Attempts have, also, been made to correlate diphtheria epidemics with the weather, for it is possible that some weather conditions are more instrumental than others in rendering children susceptible to the disease.

The average annual death rate in the United States due to diphtheria before the days of antitoxin was approximately 100 for every 100,000 population. If this ratio had continued to the present time we would be losing annually 100,000 from diphtheria. In 1900, just five years after the discovery of antitoxin, the death rate had dropped to less than one half of what it was before the days of antitoxin. Since 1900 there has been a gradual and consistent decrease.

The following results indicate clearly the effect of diphtheria antitoxin on the diphtheria death rate.

NUMBER OF CITIES WITH VARIOUS DIPHTHERIA DEATH RATES

Year	Number of cities	40 and over	20 and over	10 and over	Under 5	0.0
1890–1894	64	52	60	61	2	0
1895–1899	66	34	53	63	1	0
1900–1904	68	22	46	64	2	0
1905–1909	72	3	43	66	1	0
1910–1914	79	1	36	63	1	0
1915–1919	84	0	25	62	3	0
1920–1924	88	0	14	65	2	0
1925–1929	92	0	1	22	25	0
1930–1934	93	0	0	0	69	0
1934	93	0	0	4	76	15

In the first period, 1890–1894, there were 52 cities in the United States with an annual diphtheria death rate of forty or over and only two under five. By 1930–1934 there was not one city with an annual death rate of over 20, and 69 were below ten. In 1934 there were 15 large cities in the United States without a single diphtheria death.

Today the mortality from the disease depends largely on the locality. It is lowest in the country, for there a person is less likely to contract the disease. It is highest in the small cities where the disease is prevalent and antitoxin is not promptly administered. The average death rate from diphtheria in fourteen large cities as compared with that of the rural districts for the years 1911–1917 is given below:

DEATH RATE PER 100,000 POPULATION

Year	Registered area	Average	
		In rural districts	14 large cities
1911	18.9	15.1	20.5
1912	18.2	15.5	18.7
1913	18.8	14.7	23.4
1914	17.8	13.8	24.0
1915	15.7	12.9	19.8
1916	14.5	11.7	19.2
1917	16.5	12.3	22.7

The death rate from diphtheria in some small cities is nearly as high as it was in the pre-antitoxin days. That is, if it were not for the diphtheria antitoxin the mortality from the disease would be what it used to be in the seventeenth, eighteenth, and nineteenth centuries, as there is no indication of a weakening of the diphtheria virus. However, the cities have shown a consistent decrease even down to 1934, as may be seen from the following table in which are given the total diphtheria death rates for 88 cities from 1923–1934.

Year	Population	Diphtheria deaths	Diphtheria death rate per 100,000 population
1923	31,060,848	4,078	13.13
1924	31,722,841	3,439	10.84
1925	32,884,834	3,133	9.67
1926	33,046,827	3,106	9.40
1927	33,708,820	3,493	10.36
1928	34,370,813	3,176	9.24
1929	35,032,806	2,738	7.82
1930	35,694,802	1,827	5.12
1931	36,503,412	1,366	3.74
1932	37,084,712	1,191	3.21
1933	37,084,712	861	2.32
1934	36,777,112	821	2.23

The Cause.—The diphtheria bacillus, the organism which causes diphtheria, varies considerably in its general characteristics. These depend largely on whether the organism has been grown on laboratory media, or whether it has come directly from the throat. When grown on Löffler's blood serum it possesses characteristics definite enough, so that it can be morphologically identified, a condition rarely met in bacteriology. The size, morphology, and staining properties vary greatly with different races of the organism. They are all slender, straight, or slightly curved rods varying in diameter from 0.3 to 0.8 micron, and in length from 1.5 to 6 microns. They may occur with clublike enlargements at one or both ends, or equatorial enlargements with ends more or less pointed. Occasionally, only one end is thickened, giving rise to a long somewhat wedge-shaped rod. The club-shaped organisms are rarely seen in films made direct from the false membrane, but they often occur in cultures grown on laboratory media. Although some strains of the diphtheria bacilli stain uniformly, it is more common to find

an alternate arrangement of dark and light bands, giving them a beaded appearance, and in extreme cases making the organism resemble the streptococci. The irregularity of staining is well brought out by Löffler's alkaline methylene blue. In many

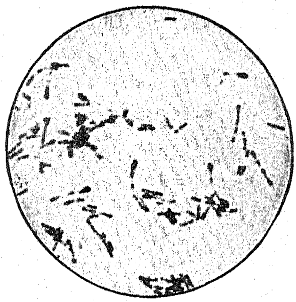


Fig. 131.—*Corynebacterium diphtheriae* (× 1100). (*Park.*)

cultures, properly stained, there can be seen situated at the equator, or near the ends, round, or oval bodies. These are stained more intensely than the rest of the body, and at one time were believed to indicate a high degree of virulence on the

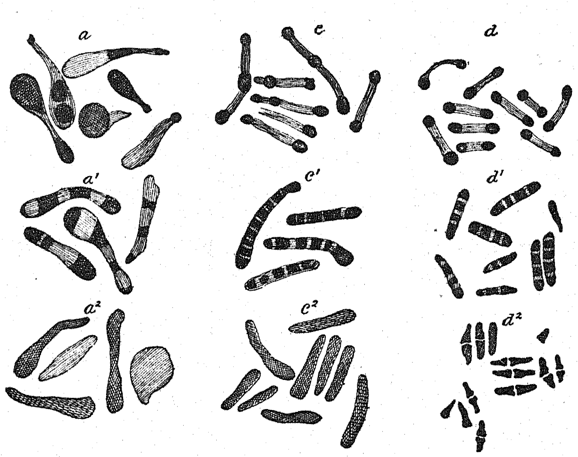


Fig. 132.—Wesbrook's types of *Corynebacterium diphtheriae.* (*From Jordan, General Bacteriology.*)

part of the organism containing them; hence, special care was used in their determination. However, today it is generally believed that they are of the same significance as the metachromatic granules in other bacteria.

The diphtheria bacteria have been divided into the following classes, according to the manner in which they take certain stains: (1) The granular type, which stains in irregular patches or blocks with the intervening part only slightly stained or unstained. This type predominates in clinical diphtheria. (2) The barred type which when stained has a striated appearance. This type is found both in diphtheria and healthy throats. It is sometimes stated that this is the sole type found in some cases of diphtheria, but Jordan considers this to be untrue. (3) The solid type which stains uniformly. Some authorities class these as pseudodiphtheria bacilli and do not regard them as capable of causing disease. However, there is some evidence that one type may change into the other. Consequently, Park and Williams consider the only practical method of differentiating virulent from nonvirulent diphtheria germs to be by animal inoculation. They have found a few strains of virulent diphtheria bacilli which fail to show a marked characteristic stain and quite a number of pseudodiphtheria organisms which show dark bodies. The important factor, however, is that all virulent strains are controlled by the same antitoxin.

The diphtheria bacillus grows on ordinary laboratory media, provided a suitable temperature is maintained (17° to 43° C., with an optimum of 37° C.). On Löffler's blood serum it produces characteristic colonies and organisms. On this medium the diphtheria bacilli outgrow the common parasitic organisms found in the mouth; consequently, this is used in the diagnosing of the disease. Diphtheria bacteria possess no capsule, are nonmotile, form no spores, but are more resistant than most nonspore-bearing bacteria. They may remain alive in dried false membranes at room temperature for several months, or for one hour at 98° C. They are killed in ten minutes at 60° C., five minutes at 70° C., and almost instantly in boiling water. They are quite resistant to disinfectants, while in the false membrane, but in the free condition they are readily killed by the ordinary disinfectants. Hydrogen peroxide is said to be especially effective against them.

Toxin Production.—The diphtheria bacillus is one of the few bacteria which produce a highly potent exotoxin. No two strains have the same power of producing toxin and some lose their ability quickly when grown on laboratory media. The one isolated by Park in 1895 is now generally used for toxin production, and it is just as powerful today as when first isolated. Toxin is readily produced by growing the organism in beef tea.

The presence of carbohydrates inhibits its production; hence, toxin is not produced in milk. Although pure toxin has never been obtained, yet, some very poisonous solutions have been prepared. The toxin is believed to be proteinaceous in nature. It is destroyed by a temperature of 75° to 80° C. and is rendered inactive by the digestive juices; but when injected into experimental animals it causes death, paralysis, or the production of antibodies, with a resulting immunity, depending upon the quantity given. The production of the toxin and antitoxin has already been considered on pages 422 to 426.

Modes of Transmission.—The diphtheria bacillus leaves the body in the secretions of the nose and throat. These secretions are, consequently, the infecting agents. The organism may be transmitted directly from one individual to another by droplets, fingers, toys, pencils, spoons, cups, handkerchiefs, and the many other objects which are placed in the mouth of the infected child and later find their way into the mouth of a healthy person. The organisms may come from the throat of active cases, recent convalescents, mild and missed cases, and carriers, and are spread by the rather quick exchange of micro-organisms from the mouth of one to that of another.

Carriers.—Löffler, in his first publication on diphtheria, referred to the fact that in the course of the examination of the throats of 20 children he found in the throat of one, bacilli which were indistinguishable from the true diphtheria germ; nevertheless, this child had never suffered with the disease. This was used as evidence that Löffler had not isolated the cause of the disease. It was learned during the nineties of the last century that individuals who have recovered from the disease, or even others who have never had diphtheria may carry within their throats the diphtheria germ. In 1893–1894 there were studied in the Health Department of New York City 752 cases with the following results:

In 43.2 per cent of the cases the diphtheria organism disappeared in 3 days.

In 26.7 per cent of the cases the diphtheria organism disappeared in 5 to 7 days.

In 11.1 per cent of the cases the diphtheria organism disappeared in 12 days.

In 9.1 per cent of the cases the diphtheria organism disappeared in 15 days.

In 7.5 per cent of the cases the diphtheria organism disappeared in 3 weeks.

In 1.4 per cent of the cases the diphtheria organism disappeared in 4 weeks.

In 0.6 per cent of the cases the diphtheria organism disappeared in 5 weeks.

From this and similar work it is often stated that the bacilli begin gradually to disappear from the body of convalescents, and by the end of a month 85 per cent of the convalescents are free of the bacilli. By the end of the second month 98 per cent are free, and the remaining 2 per cent pass into the chronic carrier state. Foreign bodies, or deformities, in the nose and throat predispose to the chronic carrier state. It has been further found that from 10 to 20 per cent of the individuals who come in contact with diphtheria patients harbor the germ in their throats. In some 80 per cent of such cases they are harboring the virulent organism, and although they do not contract the disease themselves, they are a danger to the community. Extensive studies indicate that about one in 1000 of the general population is a carrier of virulent diphtheria germs. However, among children, about 2 per cent are carriers, and about 10 per cent of these harbor diphtheria germs capable of causing the disease. Often considerable difficulty is experienced in trying to free chronic carriers of the germs. This is really one of the big problems which confront the health worker, for were it not for the carriers the disease would be much less of a problem. It is the carriers that normally keep the disease alive and greatly facilitate the spread during epidemics.

The Schick Reaction.—For some time it was not understood how the healthy individual could carry the diphtheria germ and not come down with the disease. In 1913 Schick reported a method by which could be determined those individuals who had within their blood sufficient antitoxin to protect them against diphtheria. It is made in the following manner: A quantity of diphtheria toxin equivalent to $\frac{1}{50}$ the minimum lethal dose for a 250-Gm. guinea-pig is made up to 0.1 cc. with sterile salt solution and injected subcutaneously, or preferably intracutaneously. A local reaction follows in from twenty-four to forty-eight hours if there is less than one thirtieth of a unit of antitoxin in a cubic centimeter of the individual's blood. This quantity is sufficient, under ordinary conditions, to protect against the disease. The application of this test to carriers showed that they carry the antitoxin. The test has been applied to hundreds of thousands of individuals. In one series of 20,000 the following results were obtained:

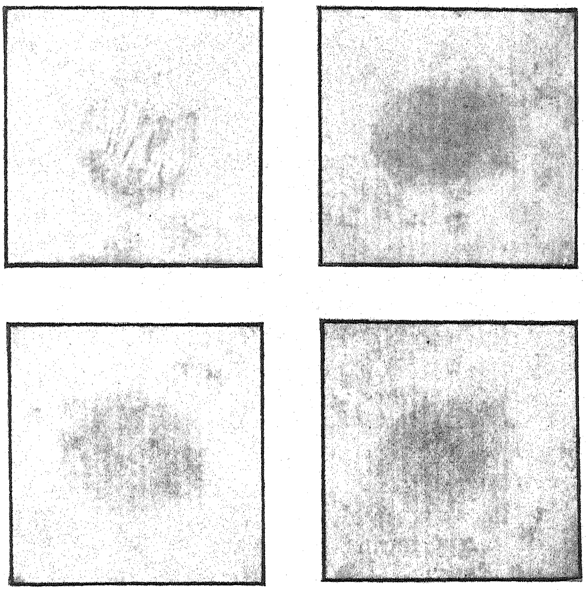


Fig. 133.—Shows four typical positive Schick reactions of varying degrees of intensity forty-eight hours after test. (*Zingher, American Journal of Diseases of Children, April,* 1916.)

Age	Average susceptible (per cent)
At birth	10
Under 4 months	15
4 to 6 months	30
6 to 9 months	60
9 months to 1 year	75
1 to 2 years	75
2 to 3 years	65
3 to 5 years	40
5 to 10 years	30
10 to 20 years	25
Over 20	20

The susceptibility to diphtheria is low at birth, but it gradually increases with the age of the individual. It becomes highest between the ages of one and two years, after which it decreases. Only about 20 per cent of the adults are susceptible to diphtheria. The results indicate that immunity goes in families, and the children of some nationalities are more immune than other children. In the city of Auburn, Sears found the positive reactions among the Polish children to be 26 per cent, among the Italian children 36 per cent, and among the English-speaking races 82 per cent; the general average for all races being 60.5 per cent. The great practical significance of the test rests on the indication that certain individuals are not immune and should receive preventive treatment.

Prevention.—During the nineteenth century, diphtheria showed a definite, although irregular increase. This was probably due to the greater crowding of individuals into densely populated centers. It showed little reduction when better sanitation was instigated. The knowledge that the disease is communicable, the identification, and the isolation of the specific germ causing the disease did not help much. Soon after the discovery of the antitoxin, in 1895, there was a decided drop in the mortality, but it had little effect on the number of cases. In New York special pains were taken to prevent convalescent carriers from being released from quarantine and to search out many healthy carriers. Still the results were disappointing; consequently, it became evident, laudable as were the campaigns against the placing of infected objects in the mouth, the careful quarantining of diphtheria cases, and the systematic use of antitoxin, that diphtheria could be controlled only when it was once learned how to effect a permanent immunity to the disease.

During the first decade of the twentieth century a number of workers had used mixtures of toxin and antitoxin to immunize lower animals, and several had suggested its use for the immunizing of children. Yet, it was not tried out until 1913, first by Behring, and later by Park. The latter found that by taking definite quantities of an appropriate mixture of toxin and antitoxin a high degree of lasting immunity could be produced in children. The perfecting of the Schick test in 1913 gave an efficient method for determining those susceptible to diphtheria, and a method of determining when the toxin-antitoxin treated child was immune.

The results of this work indicate that toxin-antitoxin treatment gives rise, in one to three months, to an immunity which lasts for at least ten years and probably life. Inasmuch as the immunity does not develop for some time after the treatment, it is valueless in those cases exposed to diphtheria. In such cases the regular antitoxin is used; consequently, it is held today by authorities that if all children were systematically tested by the Schick method and the susceptible ones given the toxin-antitoxin treatment, diphtheria could be completely eradicated. During the four years previous to the use of toxin-antitoxin on the school children of Auburn, a city in New York with a population of 36,000, the average annual death rate from diphtheria was 14, and the number of cases 104. Three years of systematic work with toxin-antitoxin reduced this to three cases with no deaths. The results obtained by Park, in his antidiphtheria campaign, which is being conducted in New York, are of the same nature, as is shown by the following results:

DIPHTHERIA CASES AND DEATHS IN NEW YORK CITY
(1919–1923)

Year	Cases	Deaths
1919	14,014	1,239
1920	14,166	1,045
1921	15,110	891
1922	10,427	874
1923	8,050	547

The cases have been reduced nearly one half and the deaths over one half in five years. These favorable results are attributed by Park to the combined efforts of the health department, medical inspectors, who were instrumental in giving immunizing antitoxins, and private physicians, as well as to the general edu-

cation of the people of the city and state of New York. It was primarily these two factors that have brought about this striking reduction in morbidity and mortality. All are agreed that we have the means of ridding communities of diphtheria; consequently, today the diphtheria problem is similar to the smallpox problem, and depends upon the education of the masses to the safety and efficiency of diphtheria immunization. The reasons it is better to prevent diphtheria with toxin-antitoxin treatment than to depend merely upon proper treatment are thus summarized by Nicoll:

"1. Antitoxin may be administered too late and in insufficient quantity to save life. Evidence of diphtheria may be so slight that a physician is not called early enough.

"2. One form of diphtheria (laryngeal) attacks the larynx, or windpipe, and may cause croup but no sore throat. The child may choke to death before medical attendance can be had.

"3. Another form, nasal diphtheria, attacks the lining of the nose, and may be regarded as a common cold until it is too late.

"4. A person who has diphtheria must undergo a period of illness and may suffer from bad after-effects, especially heart disease.

"5. Other members of the family must usually suffer inconvenience from quarantine regulations."

Antitoxin.—The sovereign remedy for the cure of diphtheria is the antitoxin, for before the days of antitoxin there were diphtheria epidemics in which the mortality was 35 per cent or over. Now, when antitoxin is given during the first signs of the disease, the percentage of recoveries is 100 per cent. The antitoxin should be administered on the very first symptoms, for every hour counts. Hilbert collected data on 2428 cases and found that the percentage of deaths varied with the day on which the antitoxin was used; the results are as follows:

Time antitoxin was administered	Percentage of deaths
First day	2.2
Second day	7.6
Third day	17.1
Fourth day	23.8
Fifth day	33.9
Sixth day	34.1
After sixth day	38.2

It has been estimated that through the use of antitoxin in the United States annually 20,000 lives are saved, and that if properly and promptly administered the annual saving in Germany alone would be 45,000 lives.

In addition to this, there are the still larger numbers who are saved from contracting the disease when given the antitoxin on exposure, as antitoxin is not only a curative but a short preventive; consequently, it is used in two sets of cases. (1) It is used in connection with those suffering with the disease; and (2) with those exposed to the disease and who have not been immunized by the toxin-antitoxin mixture. There is practically no danger from the use of the antitoxin, and when properly used it means a speedy recovery from diphtheria. Occasionally it happens that individuals, who suffer from the disease and are given the antitoxin, die, or are left paralyzed, or otherwise seriously injured. However, in no case are these effects to be considered due to the use of the antitoxin. They simply indicate that the antitoxin was administered too late, and that the disease had done so much damage before the antitoxin was given that the individual would have died or never have completely recovered anyway. When insufficient quantities of antitoxin are given the lives of the patients may occasionally be saved, but because the amounts were not sufficient to neutralize all the toxin, the individuals are left permanently injured after recovery.

CHAPTER XLII

TETANUS

Tetanus, or lockjaw, as it is commonly called, is a comparatively rare disease; nevertheless, due to its peculiar spasmodic symptoms and its high mortality, it early attracted special attention. The discovery of the causative organism intensified this interest. It is caused by an anaerobic highly resistant spore-producing saprophyte. It enters the body through a wound and elaborates, at the point of entry, a highly potent toxin which damages primarily nervous tissue.

History.—It is evident from the writings of Hippocrates and his contemporaries that tetanus as well as some other diseases that have been mentioned were prevalent and recognized before the beginning of the Christian Era. The Arabian physicians during the ninth and tenth centuries accurately described the disease, but they, like their predecessors, attributed it to sudden changes in the weather, the wetting and chilling of the body. The advancements in the field of medicine are mirrored by the interpretations placed upon this disease. It ran the gauntlet of the prevailing theories of the ages—evil spirits, improper humors, miasma, noxious effluvia, taking cold, rheumatism, and finally the germ theory. Each, at one period or another, was pressed into service to explain tetanus. About the middle of the nineteenth century the belief that it was an infectious disease commenced to crystallize. From 1868 to 1870 numerous attempts were made to infect experimental animals with tetanus by blood from men suffering with the disease. As can be readily understood today, the results were all negative. In 1884 Carle and Rattone for the first time produced the disease experimentally. They demonstrated that the disease can be transmitted from the wound of one animal to that of another. The following year Nicolaier, led by the observation that gardeners are prone to contract the disease after slight injuries to the feet and legs, produced it experimentally in a number of small animals by inoculating them with garden soil. He was able to demonstrate that in the pus of such wounds were drumstick-shaped bacilli. Four years later Kitasato recognized this enlargement of the bacillus to be

the result of a spore; consequently he heated it, killed all vegetative organisms, and thus obtained the germ in pure culture. This he inoculated into experimental animals and found that they contracted tetanus. The following year Faber discovered that a filtrate of the medium in which the bacilli had grown, but from which their bodies had been completely removed, caused the disease. About the same time Behring and Kitasato prepared an antitoxin and demonstrated its value.

Prevalence.—Inasmuch as the wounded soldier occasionally contracts tetanus, it is often stressed in relation to war. Accurate statistics are not available, yet an idea of its prevalence may be gained from the following table:

	Number wounded	Cases of tetanus	
		No.	Percentage
Crimean War	12,094 (English)	19	0.15
American Civil War	217,000	505	0.20
Russo-Turkish War	51,700 (Russian)	66	0.12
World War	27,677 (German)	174	0.62
World War	380,000 (English)	292	0.08

Among the wounded sent to England during the World War there were about 1450 cases of tetanus, slightly over 1 in 1000, and it has been estimated that were it not for the early systematic use of tetanus antitoxin there would have been 14,000 cases.

It is, however, not in war but in peace that this germ has taken its greatest toll. Before the days of aseptic surgery, and in some localities even at the present time, the organism entered the newborn infant through the umbilical wound. Among the negro babies in certain of the West Indian Islands tetanus has had a mortality as high as 50 per cent. In 1856, 4 per cent of all deaths in Charleston and 3.7 per cent of all deaths in New Orleans were caused by this disease. It has often taken a terrible toll among the negro children. In Venezuela it is one of the chief causes of infant mortality. One author states that at one time one fourth of all the children born in Rio de Janeiro died from tetanus.

Until the gospel of a sane and safe Fourth of July had been

vigorously preached, there was, as is shown in the following table, the annual July crop of deaths from tetanus in the United States:

Year	Number of cases of tetanus
1903	406
1904	91
1905	87
1906	75
1907	73
1908	76
1911	10
1916	0

As a result of the vigorous campaign waged by the American Medical Association for sane Fourth of July celebrations, and the early use of tetanus antitoxin this loss has been checked.

Occurrence.—The tetanus bacillus is widely distributed in nature. Its normal habitat appears to be the alimentary tract of herbivora; hence, it occurs in the droppings of horses, cows, and many other animals. It frequently occurs in the feces of man, and it is usually stated that it is more prevalent where individuals are around horses. The following table illustrates the extent to which it has been recovered from human feces:

Investigator	Percentage	
Pizzini	5	
Tullach	33	(Soldiers returning from France)
Tullach	16	(English civilians)
Ten Broeck and Bauer	34.7	(Chinese)
Ten Broeck and Bauer	26.5	(Chinese)
Buzello and Rahmel	40	(Germans)
Kalm	12.5	(New York)
Bauer and Meyer	24.6	(California)
Bauer and Meyer	21	(Country at large)

The number of determinations in some cases was low; consequently, the error is large if one tries to apply this to mankind as a whole, but it is suggestive and seems to indicate that about one fifth of the population are tetanus carriers. As the results indicate, there is a wide variation depending on the locality.

Individuals in some districts would ingest the tetanus spores oftener than would individuals in other districts. The results establish the following points: (1) Human carriers are not limited to persons associated with horses and cows; and (2) human feces may play a prominent part in infecting soil.

The organism is widely distributed in soil, especially garden soils. It has been obtained from hay, clothing, the skin, old masonry, fire crackers, gun wads, gelatin, and catgut. LeDantic reports having found it on the poisoned arrows of the natives of the New Hebrides, who obtained it by smearing their arrowheads with the material found in the burrows of crabs. In a few cases it has accidentally found its way into vaccines and serums with fatal results. However, the precautions taken at the present time in the manufacturing of these products will most probably prevent its recurrence.

Recently a number of fatal cases of tetanus have resulted in the United States from the use of bunion pads as vaccination dressings. Laboratory tests showed 25 per cent of the bunion pads examined to carry tetanus bacilli. Deaths from this source are entirely unnecessary, for even superficial wounds should be dressed and covered with sterile dressing. Furthermore, the use of a shield of any form in vaccination is to be discouraged, since it tends to prevent evaporation, retains the moisture, heat, and exudates, and consequently, softens the vesicle and renders conditions better for bacterial invasion.

Tetanus bacilli are more prevalent in warm than in cold districts, although Iceland at one time suffered severely from this disease. The soils of the Atlantic States, as well as the soils of Flanders and France contain great numbers. Apparently, wherever the feces of man and the lower animals reach the soil large numbers of tetanus bacilli may be expected.

Properties.—The tetanus bacilli are long, comparatively slender, rodlike organisms with rounded ends. They measure from 0.3 to 0.5 micron in diameter and from 2 to 4 microns in length; usually occur singly, or in pairs, although long chains sometimes occur in cultural media; are slightly motile, with flagella surrounding the body; have no capsule but produce spores. The spores are produced in the end of the mother cell and have a diameter greater than the bacilli; consequently, while they are in the body of the mother cell they give rise to a drumstick-shaped organism which at times resembles minute pins. They are highly resistant and survive in flowing steam for forty minutes, and occasionally for sixty minutes. They may

survive a dry temperature of 125° C. for twenty minutes and remain alive in dry soil for many years. Henrijean found them to germinate after eleven years of drying. A 5 per cent solution of carbolic acid destroys them in about fifteen hours, but if 0.5 per cent hydrochloric acid is also added, they are killed in two hours. Mercuric chloride (1 to 1000) kills them in about three hours, and a 1 per cent solution of silver nitrate in one minute. Iodoform is said to be very effective in preventing germination.

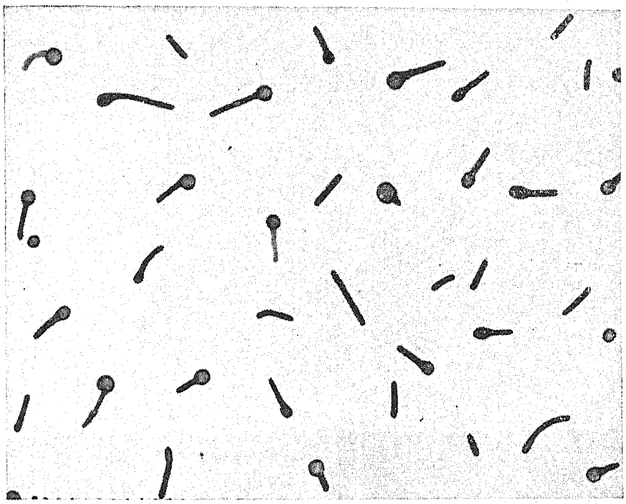


Fig. 134.—*Clostridium tetani* showing spores. Eleven-day culture in plain agar stab, stained with gentian violet. (*From Ford, A Text-book of Bacteriology.*)

The tetanus bacillus under anaerobic conditions grows well in ordinary laboratory media. If cultured with certain aerobes it grows well even though oxygen has access to the surface of the media. Its optimum temperature is 37.5° C. It stains readily with the common aniline dyes.

Pathogenicity.—Mice, guinea-pigs, rabbits, horses, cattle, goats, and men are highly susceptible to tetanus. Rats and birds are less so, and fowls scarcely at all. The cold-blooded animals are immune, although it is stated that they may be infected by raising their temperature. It is generally stated that if 1 Gm. of horse requires 1 part of toxin to kill, then 1 Gm. of guinea-pig requires 6 parts; 1 part of mouse, 12; of goat, 24; of dog, 500; of rabbit, 1500; of cat, 6000; and of hen, 360,000. It

is surprising to find that the tetanus bacilli multiply in the alimentary tract of the horse, yet a quantity of toxin sufficient to kill a hen would suffice to kill 500 horses.

The tetanus bacilli do not circulate in the blood but elaborate their toxins at the point of entry. This, in turn does the damage to the various tissues of the body, especially the nerves. Pure cultures of the organism, free from toxin, when injected into experimental animals are usually rather quickly destroyed. But if the tetanus bacilli, together with some of their toxin, or even other irritants are used, tetanus is likely to follow. The likelihood of tetanus varies with the wound. Deep or infected wounds, and those in which the surrounding tissue is destroyed, or even greatly damaged are more likely to develop tetanus if the organism is present, than clean-cut, nonsuppurating wounds. However, the severity of the wound does not influence the likelihood of tetanus. It may result from pin pricks, nail punctures, insect bites, vaccination, and many other mild injuries. It is evident that the likelihood of tetanus is always present in dirty wounds. Covering even superficial wounds with adhesive tape, or collodion produces anaerobic conditions that may become favorable for the development of the tetanus bacilli.

Toxin.—The tetanus bacilli in appropriate media elaborate a very potent toxin. The growth of the organism in beef tea free from glucose has resulted in a solution so highly toxic that 0.000,000,5 cc. will cause the death of a mouse, and purified solutions have been obtained one hundred times as toxic. It is estimated that the toxin produced by the tetanus germ is twenty times as poisonous as dried cobra venom. The potency of the toxin formed varies with different types of organism, but fortunately all are neutralized by the same antitoxin. Tetanus toxins are composed of several poisons. The best known are tetanolysin which destroys cells, and tetanospasmin which causes the spasms. These toxins are destroyed by heat, sunlight, and oxidation but may be precipitated and kept in a vacuum in the dark for long periods. If taken by the mouth the toxin is harmless, but if injected into susceptible animals it causes severe nerve injury. The time elapsing between the administering of the toxin and the appearance of the symptoms in mice has been found to vary with the dose.

It appears that time is required for the toxin to reach and injure the tissues and this cannot be obliterated by even massive doses. In this respect bacterial toxins differ from many other poisons.

1 fatal dose	Symptoms	4 to 7 days
2 fatal doses	Symptoms	2 to 3 days
10 fatal doses	Symptoms	36 to 48 hours
300 fatal doses	Symptoms	20 hours
3,000 fatal doses	Symptoms	12 hours
30,000 fatal doses	Symptoms	10 hours
90,000 fatal doses	Symptoms	9 hours

The incubation period in man, counting from the time the wound is received until symptoms appear, has been found to vary from two days to one year. However, it is usually from four to seven days.

Antitoxin.—The tetanus antitoxin is more effective in preventing than in curing tetanus. Although, according to some authorities, its proper use, even after symptoms have developed, has reduced the mortality from about 80 to 40 per cent, its failure to always effect a cure is probably due to the fact that the toxin has a greater affinity for the nervous tissue than it has for the antitoxin. It was shown that if a mixture of toxin and antitoxin was made before injecting into an animal, twelve fatal doses were neutralized by 1 cc. of a 1:2000 dilution of antitoxin. If the antitoxin was administered four minutes after the toxin, 1 cc. of a 1:600 dilution was required; if eight minutes after, 1 cc. of a 1:200 dilution; if fifteen minutes after, 1 cc. of a 1:100. Hence, if tetanus is to be prevented it is desirable that the antitoxin be administered at the earliest date possible after the wound is received.

Prevention.—The prevention of tetanus rests upon three factors: (1) The prevention of wounds in so far as possible, for it should always be borne in mind that even the breaking of the skin is a welcome to micro-organisms. Unnecessary wounds from nails and other objects occur entirely too often. (2) All dirty wounds should be carefully cleaned and then properly dressed. During the war the best method of cleansing wounds was found to be with the sugeon's knife. All dirt, bits of clothing, foreign material, and destroyed tissue should be removed. The wound should be dressed in such a manner that air has access to it. (3) When dirty, highly infected wounds of such a nature that tetanus may result are received, antitoxin should be administered by a competent physician.

CHAPTER XLIII

TYPHOID

An examination of mortality tables convinces one that some parts of the human body are more vulnerable to the attacks of micro-organisms than others. Approximately 30 per cent of the annual deaths in the United States are caused by the breaking-down of the respiratory system, and 23 per cent to a breaking-down of the gastro-intestinal system. Although this is not completely due to microbial activities it is evident that these two systems are much more exposed to the vicissitudes of the environment than are the other systems. Most bacteria enter the body by the respiratory or digestive routes and often locate in one system or the other and cause the breaking down of the whole body. Tuberculosis and pneumonia have already been considered as types of the respiratory diseases. Typhoid will now be taken to represent the gastro-intestinal group of diseases. This group is composed of typhoid, paratyphoids, cholera, and the dysenteries, all of which are quite closely related. Practically all of these diseases are peculiar to man and most prevalent during hot weather. The causative organisms enter the body through the mouth and leave in the alvine discharges. They breed primarily in the human body; consequently, man is the fountain head. Fingers, flies, and food play a prominent rôle in their spread. In the sense that these diseases have yielded to good sanitation to a greater degree than most other communicable diseases, they may all be classed as filth diseases.

History.—The disease now recognized as typhoid or enteric fever was for centuries confused with other lasting fevers. Even today it may be mistaken for septic fever, typhus fever, malaria, tuberculosis, and other lasting fevers unless bacteriological means are used in its diagnosis. Hippocrates, in writing on epidemics, describes certain diseases which he finds are generally accompanied by delirium, disturbances of the bowels, great emaciations, and a fever which sometimes lasts for forty days. He also notes that these diseases are more prevalent at some seasons than at others. The first attempt to distinguish typhoid fever from typhus fever was made by Thomas Willis in 1659,

who found that typhus fever was more contagious than typhoid, caused more eruptions on the body of the patient, and was accompanied by fewer complications than typhoid. From this date on, there were occasionally individuals who believed that typhus and typhoid fevers were two distinct diseases; but even as late as the nineteenth century all continued fevers accompanied by delirium were grouped under the general head of typhus fever, and it was not until the nineteenth century with the work of the French clinicians that the fact that there are two distinct diseases was established. The term typhus was retained for the disease in which there are no intestinal lesions. The term typhoid, which means resembling typhus, was applied to the disease accompanied by intestinal lesions. Pettenkofer had taught that the filth in soil undergoes a ripening process in which the virus of typhoid is elaborated, and the actual spread of the disease he considered due to the fluctuation of the ground water. In 1856 William Budd commenced the publication of a series of articles which laid the foundation for our present knowledge. In these he contended that typhoid is contagious, that the causative organism is found in the dejecta of the patient, and that carriers play a part in its spread. The Typhoid Commission, which functioned during the Spanish-American War, definitely proved that the disease is contagious and may be carried by flies, and that it was typhoid, and not the so-called "typhoid malaria," that was "playing hob" with our troops.

The year 1880 marks the beginning of our bacteriological knowledge of typhoid. Eberth, Klebs, and Koch each made independent contributions at this time concerning the causative organism. Four years later Gaffky grew the organism in pure culture but failed to produce the disease by feeding cultures to experimental animals. However, since then enough evidence, some gained from accidental laboratory infections, has accumulater to fully demonstrate that the *Eberthella typhosa* (*Bact. typhosum*) is the specific cause of typhoid fever. Other work has shown that the closely related micro-organisms, *Salmonella paratyphi* (paratyphoid α and paratyphoid β), give clinically similar, but bacteriologically, distinct diseases. Several bacteriological methods for diagnosing the various fevers have also been developed, as well as a highly successful preventive vaccine.

Prevalence.—In the past typhoid has been one of the great causes of death and disability, and in rural districts it is a great factor even at the present time. The extent of this disease in the United States in 1900 has been estimated at 353,790 with a

death rate of 35,379. By 1914 the number of cases had diminished to 198,000 with a death rate of 19,800. In 1933 there were almost 65,000 cases and the death rate was probably about 6500. This is favorable but entirely too high for this preventable disease. The mortality from typhoid fever commenced to drop in many civilized countries in 1880 and has shown a gradual and persistent decrease ever since. The decrease has been more rapid in foreign countries than it has in America, as may be seen from the following figures for 1910:

	Population	Average mortality per 100,000
33 largest European cities..................	31,500,000	6.5
57 largest American cities..................	21,000,000	19.59

In this disease the United States, as compared with foreign countries, has been extremely backward in applying recognized methods of sanitary prevention. In 1927 the mortality of this disease in the United States in general was not large (5.5 per

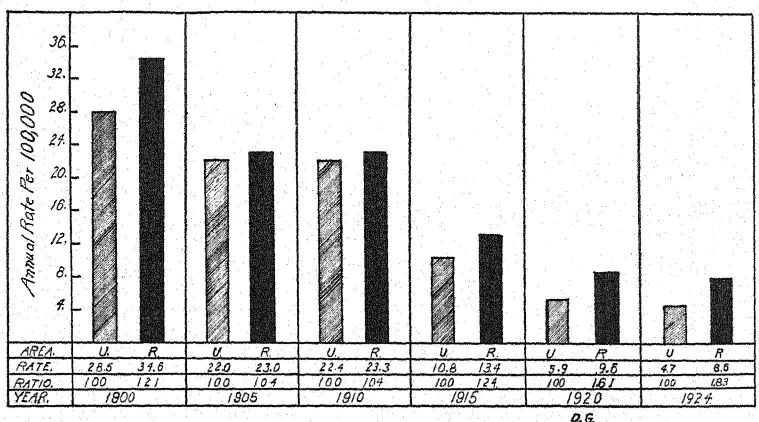


Fig. 135.—Comparative incidence of typhoid in city and rural district.

100,000), but when this is compared with the average typhoid death rate of the cities (3.07), it is evident that much remains to be done in the rural districts. According to Rosenau: "In recent years there has been scarcely enough typhoid fever in

our large metropolitan centers to furnish material to teach medical students." Typhoid fever within recent years has passed from a disease of the city to a disease of the country and in some places is referred to as a vacation disease, because of the fact that some contract it during their vacations in the country and carry it back to the city.

Appalling as have been the losses from typhoid in civil communities, they have been still greater in the armies. This is shown by the following mortality statistics from Munich—city and garrison.

	Munich city	Munich garrison
1855–1869	204	840
1876–1881	57	190

It is estimated that during our Civil War there were 75,361 cases of typhoid in the army with 27,056 deaths. Typhoid fever accounted for 65 per cent of the total deaths in the Franco-Prussian War. The typhoid losses in the Spanish-American War far exceeded those from the enemies' bullets. Similar conditions existed in the British South African War. In the recent World War, as a result of vaccination, typhoid fever played an insignificant rôle.

The economic loss due to typhoid fever is great. It is a disease which usually attacks robust individuals who are in the prime of life. It is estimated that fully 50 per cent of its victims are between the ages of fifteen and twenty-five years. It is a long-drawn-out disease which not only keeps its victims from his work for long periods, but necessitates the services of others to care for him. Whipple has estimated that each typhoid death is a loss of $6000 to the community. At this rate, the cost in 1900 was over $200,000,000 and in 1933 between $30,000,000 and $40,000,000!

Causative Organism.—Typhoid is often referred to in the plural as there are three similar organisms causing similar diseases: *Eberthella typhosa* (*Bact. typhosum*), *Salmonella paratyphi* α (*Bact. paratyphosum* α), *and S. paratyphi* β (*Bact. paratyphosum* β). Morphologically and physiologically the organisms are similar and belong to the colon-typhoid group. Often too, the diseases produced by these organisms are so

similar that they can be differentiated only by bacteriological methods. Singularly too, an attack of one does not protect against an attack of another.

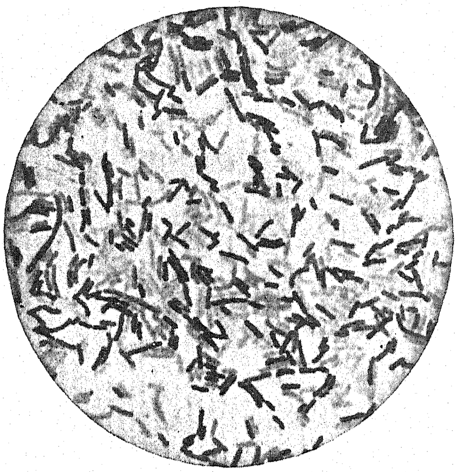


Fig. 136.—*Eberthella typhosa.* (*Hicks.*)

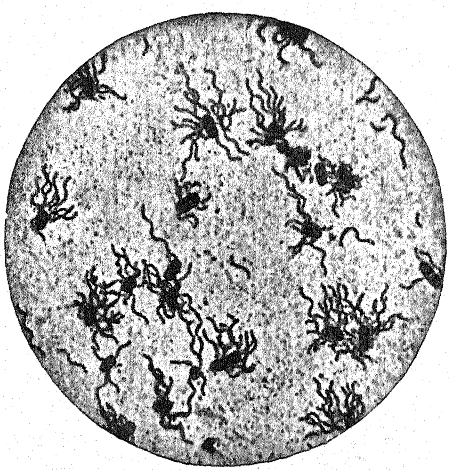


Fig. 137.—*Eberthella typhosa* showing flagella. (*Fränkel and Pfeiffer.*)

Paratyphoid seldom occurs as an epidemic, and usually has a shorter incubation period and a lower mortality than is the case with typhoid. The organisms of these diseases differ in their ability to ferment certain sugars.

The *typhoid bacilli* may be obtained from the rose spots, circulating blood, feces, and urine of the patient during the time he is ill with the disease and from certain of his organs after he is dead. They stain readily with the ordinary aniline dyes, and morphologically are short plump rods, varying in length from 1 to 3 microns and in diameter from 0.5 to 0.8 micron. Usually when grown in a liquid they are actively motile and somewhat longer than when they are grown in solid media. These characteristics may be lost temporarily in old cultures or when the medium is too alkaline. Under appropriate conditions capsules may be demonstrated.

The organisms grow well on ordinary laboratory media but less readily than *Escherichia coli.* Their optimum temperature is that of the human body, but they will grow through a range of from 8° to 44° C. A temperature of 60° C. for ten minutes, or 100° C. for three minutes kills the bacilli. They are highly resistant when in feces. They withstand cold well, survive in ice, and especially in ice cream, for long periods, and they may survive in water from fifteen to twenty-one days, although they usually disappear sooner. They multiply rapidly in milk but not in water, even though it may be heavily laden with organic matter.

Occurrence.—The great reservoir of the typhoid bacilli is man himself, and even when found outside the human body, they can be more or less directly traced to the discharges of a typhoid patient or convalescent. From man they may find their way into soil, water, milk, food, or onto anything handled by man. They multiply in milk and certain foods, but their multiplication outside the human body is very limited. They find their way into natural water with sewage, but the times they have been isolated from water are few. The organisms may survive for weeks, but do not multiply in the fecal matter of privy vaults and polluted soil.

Pathogenicity.—The typhoid bacilli naturally attack only man. They enter through the mouth, cause ulcers in the intestines, and pass from the intestines into the blood stream. The period of incubation varies from ten to twenty-one days, during which time no symptoms are present. The disease often begins with a headache, listlessness, pain in the legs and back, followed by the characteristic fever. The fever is slight at first, but gradually increases from day to day until it reaches a maximum of 103° to 104° F., and sometimes higher. This is followed by a similar steplike decline. In the majority of cases rose-red spots

appear on the abdomen, and the patient goes into a delirium. It is as if a cloud were cast over the mind. Vaughan very vividly described the effect as he saw it during the Spanish-American War: "Sometimes their eyes turn toward me or toward a comrade. In some, the eyes are full of meaning, bespeaking a brain in action; in others they are dull and staring, indicating benumbed or comatose intellectuality. Some faces show the hectic flush of fever; some the pallor of approaching death; some of the figures are full and rounded, showing but slight departure from health; others are wasted and skeleton-like; some are motionless; others are picking at the bedding, or plucking imaginary objects from the air. All are prostrate on cots, but some are attempting to rise and unreasonably impatient of restraint. The pictures are not all silent ones; some are gentle in speech; others are violent and denunciatory; some are muttering in low delirium; others are shouting in wild mania."

Typical typhoid has been produced in the chimpanzee by feeding milk, or broth cultures of the germ. A form of the disease has been produced by feeding carrots smeared with typhoid bacilli to fasted rabbits. Most animals are unharmed when fed the typhoid germ, but many succumb when the organisms are injected into their tissues or blood streams. However, the resulting disease cannot be considered as typical typhoid. Fear is sometimes entertained that cows drinking typhoid infected water, may thus infect the milk, but such fears are groundless. The only way in which milk can be infected is from the infected surface of the animal's body. Milk is usually infected by a human carrier.

Routes of Infection.—Typhoid bacilli, under ordinary circumstances, commence to diminish immediately on leaving the human body. This phenomenon aids greatly in limiting typhoid, for, when the germs live under unfavorable conditions, each successive link in the chain which connects the first typhoid case with the second generally tends to become weaker and to diminish the danger of infection. It is, therefore, not surprising to find that most cases come from the rather rapid transfer of the excreta of the body of one person to that of another. It is impossible to measure exactly the magnitude of each method in typhoid distribution, but the following results are instructive, as they give the percentages of typhoid attributed by various authorities to different causes:

	Contact	Water	Milk
Chapin	10–40	10–15	0.2
Frosch	65	23.7	
Kober	....	80	
Rosenau	6–17	35	10
Spatta	33	40	25
Whipple	30		

It is important to note the extremely high values assigned to contact in true typhoid which the laity considers a "noncontagious disease." It is in reality a communicable disease which is conveyed from individual to individual only less often than the so-called "contagious diseases," because of the manner in which the organism leaves the body of the diseased. The fact that the organism is given off with the urine and feces considerably lessens the likelihood of its reaching the hands or food of man. There is, consequently, much less danger of being infected by these means than in the case of those diseases whose germs leave the body in the secretions of the nose and mouth.

Gay mentions the following routes by which the typhoid germs may be tranferred:

1. Fingers, or utensils, to mouth. (This is the route of direct contact.)
2. Fingers, to food, to mouth.
3. Fomites, fingers, food, to mouth.
4. Flies, food, to mouth.
5. Fomites, flies, food, to mouth.
6. Water to mouth.
7. Water, food, to mouth.
8. Soil, water, or food, to mouth.

Contact.—The disease may be spread by the physician, nurse, and other individuals who care for the ill. This may readily be accomplished by infected fingers, thermometers, tongue depressors, cups, spoons, contaminated towels or clothing. That this occurs is borne out by the fact that physicians and nurses are more likely to contract the disease than others. It often spreads rather rapidly in rural districts where neighbors take turns caring for the ill. This, as pointed out by Vaughan, was the main method by which the disease spread during the Spanish-American War. "Before us every morning regiments were drawn up and so many men detailed from the ranks to serve in the hospitals as orderlies for the day. We followed these men to the hospitals and saw them handling bedpans in their awkward,

ignorant way, often soiling their hands, as well as the bedding, floors, and ground. At noon they went to lunch, most of them without washing their hands, to say nothing of disinfecting them, handling their food and passing it to their comrades. A like demonstration was repeated at supper. The next day a repetition of this cycle was repeated." Such procedures caused 20,000 American soldiers to contract the disease, which clearly indicates that an inexcusable lack of cleanliness, resulting either from carelessness or ignorance was prevalent. Contact cases are said to be especially severe. This may be due to the fact that the virulence of the micro-organisms has had no opportunity to decrease outside of the body, as is the case when the individual becomes infected indirectly. There is also the possibility of the infecting dose being large in contact cases.

Flies.—It had been suggested on several occasions that the common fly was probably an agent in the spread of typhoid, yet this was not generally understood until the report of the Typhoid Commission appeared in 1898. In this it was shown that flies swarmed over infected matter, becoming soiled with it, and then visited and fed on the soldiers' food. Lime was sprinkled over the latrines, and a few minutes later flies, with their feet whitened with it, were walking over the food. It was further shown that individuals who ate in screened rooms were less likely to contract the disease than were others. Later, Hamilton completed the evidence against the fly by obtaining from its body typhoid germs. It is now known that flies may convey typhoid germs either on their bodies or in their digestive systems, as the typhoid bacilli may pass the alimentary tract of flies without reduction in virulence. The part played by flies varies widely. It is of small importance in well-sewered cities but often of first magnitude in rural districts. Even aside from the spread of typhoid fever, the fly is a dirty unmitigated pest which should be destroyed.

Milk.—Milk may not only convey the organisms from individual to individual, but it offers a suitable medium for their multiplication. Quite extensive outbreaks of typhoid have occurred in both England and America as a result of infected milk. In 1908 an epidemic occurred in Boston in which there were 410 cases reported. All were traced to the milk which was believed to have been infected by an individual tasting it. Milk-borne epidemics have certain characteristics which set them off rather sharply from those having other origins.

1. The disease follows the route of the milk man. It is at times possible to plot his route from the cases of typhoid.

2. The better class suffer more than do others, as they use more fresh milk.

3. Those who drink milk are chiefly affected; consequently there is a greater proportion of cases among women and children.

4. The outbreak is explosive in nature, and there is often more than one case in the same household.

5. The incubation period may be shortened, probably because of the large quantity of infected milk consumed.

6. The disease is usually milder than when it comes from contact or water. This is probably because growth in milk decreases the virulence of the bacilli.

The remedies against milk-borne typhoid are: (1) Exclude, so far as possible, all typhoid carriers and mild cases from the dairy; and (2) properly pasteurize all milk in the final sealed containers.

Water.—It has already been shown that water plays a prominent part in the spread of typhoid. In the past the reduction of typhoid has been primarily a result of improved water supplies. In rural districts, however, much still remains to be done in this regard. Water-borne typhoid can be attacked by carefully guarding domestic water supplies against human sewage. This becomes more difficult as the population increases; consequently, it is becoming more necessary each year that water be purified or treated in some manner before it is used.

Food.—Many foods have been incriminated as carriers of typhoid. The ones which are the most dangerous are those that are eaten raw, especially if they have been produced in sewage-polluted soil or water. Lettuce, radishes, celery, and watercress have all contributed their quota. When once infected ordinary washing is not sufficient to free them from the typhoid germ.

Oysters, mussels, and shellfish, if grown in polluted waters and eaten raw, may cause typhoid fever. During 1924–25 there was a widespread epidemic caused by infected oysters. This involved at least 1500 cases and over 100 deaths. The outbreak was traced to oysters produced in West Sayville, New York, and it was proved that although the typhoid bacilli do not multiply in oysters they may survive in them for several weeks. Inasmuch as oysters are occasionally produced and often fattened in sewage-polluted water there is only one safe procedure; namely, to eat only well-cooked oysters.

The seasonal prevalence of typhoid often gives an index of the possible cause. Fly-borne typhoid occurs only in the warmer months of the year; water-borne usually in the fall or spring; typhoid due to vegetables and fruits correspond with the season in which these are most used; whereas milk outbreaks may occur at any season of the year.

Carriers.—Typhoid fever is a septicemia. The germs pass from the intestines into the blood stream and in this manner visit every organ of the body. It is, thus, not surprising to find that they occasionally locate in some part of the body and later become a menace to the host and especially to the community. The bacilli disappear from the blood several days before the temperature of the body becomes normal, but even in favorable cases they remain in certain parts—bone marrow, gallbladder, and intestines—for several days after the temperature is normal. Occasionally some of them remain in these organs for days, weeks, or even years; and as a consequence the patients become bacilli carriers. The germs which have located in the bone marrow may later cause diseases of the bones; those in the gallbladder may give rise to gallstones, for typhoid bacilli have frequently been obtained in pure cultures from gallstones. Such bacilli carriers are not as great a menace to themselves as they are to the community. The gallbladder serves as a reservoir and a point for multiplication, and from here the organisms are poured into the intestines and appear in the feces.

Typhoid carriers are usually divided into three groups:

Group I.—Precocious or incubation carriers. These are individuals who have ingested the bacilli and are eliminating them before the symptoms of the disease have appeared. There are some individuals who excrete the germs before they themselves develop the disease; usually this is only for a few days, although there are cases on record in which this has occurred for several weeks before the disease has manifested itself.

Group II.—Persons who have recovered from the disease but continue to carry the bacilli. These are of two classes: (1) Convalescent carriers—persons who eliminate the bacteria up to three months after recovery; and (2) chronic carriers—persons who eliminate the bacilli for longer periods than three months.

Group III.—Healthy or passive carriers. These are persons who eliminate the bacilli, but have no history of having had the disease.

After a careful study of all available data, Gay concluded that of all the individuals who recover from typhoid fever from 4 to

5 per cent become chronic carriers. From this he concludes that in the United States about 7500 carriers are annually added to the number already in existence. The annual reduction in typhoid which has occurred during recent years has also resulted in a corresponding decrease in carriers. Many authorities believe that there are approximately three healthy carriers to every 1000 persons in this country. This means that the United States still has an army of about 300,000 carriers, and Gay considers it conservative to attribute one fourth of all cases of typhoid fever to carriers.

The actual danger of a given carrier varies with his occupation. Lumbermen, miners, stenographers, and the like may be of little danger, but cooks, milkmen, and all individuals engaged in the production, handling, and vending of food, especially that which is eaten raw, are a great menace. The examination of about 30,000 food handlers in the army during the World War revealed that less than 0.1 per cent of the healthy males were carriers. The danger in civilian life is greater than these figures indicate, for there are about five times as many female carriers as male. Most carriers are adults.

A remarkable instance of a typhoid carrier was the one investigated by Soper and known in history as "Typhoid Mary." Even when detected she refused to cooperate with the investigators in furnishing information concerning her past history, but definite information was obtained back as far as the year 1901. At that time "Typhoid Mary" whose real name was Mary Mallon, was employed as a cook. A visitor developed typhoid ten days after visiting the family in which she worked. In 1902 she obtained another position. Two weeks later the laundress was taken ill with typhoid. Soon seven members of the household were ill with it. In 1904 she went to a family in Long Island; within three weeks after her arrival four servants were ill with the disease. In 1906 she went to another family. Soon six out of the eleven inmates were ill with the disease. Here for the first time she was suspected but was not proved to be a typhoid carrier, until she had entered another family and infected the laundress. Mary was tested and found to be excreting typhoid germs in great numbers. Soper writes: "The implication was plain; the cook was virtually a living culture tube in which the germs of typhoid multiplied and from which they escaped in the movements from her bowels. When at toilet her hands became soiled, perhaps unconsciously and invisibly, so when she prepared a meal, the germs were washed and rubbed

from her fingers into the food. No housekeeper ever gave me to understand that Mary was a particularly clean cook." She was detained for some time by the New York Department of Health, but later paroled on the promise that she would not take employment as a cook. She soon broke her parole and nothing was heard of her for five years. An outbreak of typhoid fever in the Sloane Hospital for Women revealed the fact that "Typhoid Mary," under the name of Mrs. Brown, was doing the cooking. Since then she has been virtually a prisoner. This girl was proved the sole cause of 51 cases of typhoid fever in ten outbreaks. Inasmuch as her whole history is not known she may have been the cause of many others.

Another destructive outbreak in Hanford, California, was investigated by Sawyer. It resulted in 93 cases of typhoid fever with a number of deaths. The vehicle of the infection was a large pan of spaghetti prepared by a carrier who gave no history of having had typhoid. The spaghetti was baked after it had been infected, but laboratory test showed that the baking incubated the bacteria instead of destroying them.

Many means have been used in attempts to cure typhoid carriers. These have met with only partial success. Consequently in most cases when typhoid carriers are detected, they are made to promise that they will not engage in work where they are required to handle food for others, and that they will keep the Health Department of the city informed of their presence.

Typhoid Vaccination.—Serums have been prepared as curative agents in typhoid fever, but the efficiency of these has not yet been proved. On the other hand, very efficient preventive vaccines have been prepared. The value of these has been fully established in both civil and military life. The results in military life are especially convincing, as the following table shows.

TYPHOID IN THE U. S. ARMY (CASES PER 100,000 MEN,

	Cases
1901 No vaccination	674
1905 No vaccination	314
1909 Voluntary vaccination	335
1910 Voluntary vaccination	243
1911 One-half Compulsory	85
1912 Compulsory	31
1914 Compulsory	7.5
1915	2

In 1898 during the Spanish-American War, there were 4422 cases of typhoid and 248 deaths among 10,759 men. In 1911 there were 12,801 vaccinated men held in San Antonio, Texas, where typhoid fever was prevalent among the civilians, but only one case developed among this body of men. In the army of the Spanish-American War there was one case of typhoid for every seven men, and one death for every 71 soldiers, whereas during the World War the U. S. Army had only one case for every 3756 soldiers and one death for every 25,641 soldiers.

Prevention.—Typhoid fever can be prevented in two ways: First by destroying the typhoid bacilli; and second, by preventing the bacilli from reaching the body of other human beings. They leave primarily in the feces and urine; consequently, these should be disposed of under all conditions, so that they do not reach food and water. This requires properly constructed sewer systems, and where toilets must be used they must be properly screened. Water should come from deep wells, springs, streams, or reservoirs which are protected from human pollution and if there is a suspicion of pollution the water should be chlorinated. Milk should be pasteurized, and food likely to convey the disease cooked. Every individual should learn and practice general rules of hygiene, such as the washing of the hands before meals and always after visiting the toilet. Every individual should treat himself as a possible carrier, in so far as personal cleanliness is concerned.

Individuals ill of typhoid, and also the recent convalescent, must be considered as typhoid incubators and special precaution taken to disinfect their excreta. Typhoid differs from most communicable diseases in that it yields to sanitary measures. It is in reality a filth disease, and absolute cleanliness insures the absence of typhoid fever. Furthermore, every case of typhoid prevented means the elimination of a future carrier; hence, as the number of active cases decreases the number of carriers is reduced and the problem becomes less acute. Wherever individuals must associate with persons ill of the disease, active carriers, or live where food and water are polluted, the only safeguard is vaccination. This is not advocated for the population as a whole, for: (1) The disease in times of peace and in the absence of epidemics can be controlled by sanitary measures; and (2) immunity by typhoid vaccination is comparatively short and must be repeated every few years.

CHAPTER XLIV

INFECTIONS COMMON TO MAN AND LOWER ANIMALS

MAN differs from the lower animals in many respects. An intrinsic difference between the two is to be found in the relationship to bacteria. Many micro-organisms attack only man, and invade only human tissues; while others attack only the lower animals. However, there is a limited number that attacks both man and the lower animals. Typhoid, cholera, measles, and many others are peculiar to man. Canine distemper, chicken, and hog cholera are limited to the lower animals. Anthrax, glanders, rabies, and bovine tuberculosis are diseases which have been known for ages as common to man and his domestic animals, and as research progresses new diseases and new relationships of old diseases are being discovered. Tetanus and tuberculosis, both diseases common to man and the lower animals, have already been considered. In this chapter consideration will be given to anthrax, glanders, Malta fever, foot-and-mouth disease, and tularemia; and in the subsequent chapters to plague, and rabies. Most of these are primarily diseases of the lower animals and only secondarily of man; consequently, it is evident that their eradication in man rests upon their control in the lower animals.

Anthrax.—Anthrax is primarily a disease of the herbivora. Cattle and sheep are especially attacked, and frequently horses, hogs, and goats. In cattle it is a rapidly developing, very communicable, and highly fatal disease. The preliminary symptoms are rather indefinite; consequently, it can usually be recognized mainly by its very rapid development, and the appearance of the internal organs after the death of the animal. Cattle die of it in two to five days. The blood is dark, and thick, and the affected animals have an enlarged spleen. Because of this last characteristic the disease is sometimes spoken of as splenic fever.

Man is susceptible to anthrax and can contract it through association with diseased animals, or through the use of products from infected animals. The organism may enter the body through feeding or inhalation. A break in the continuity of the skin or mucous membrane invites infection when the organism

is present. When it enters the body of man through the alimentary tract in infected food or water, the resulting disease is highly fatal. Wool pickers and workers in horse hair and other raw products, which may come from infected animals, contract the pneumonic type of the disease. This is known as woolsorters' disease and is frequently fatal. The immunity of man is fairly high, and when it enters through an abrasion the result is usually local. This may occur on the hands of butchers, veterinarians and others. It often occurs on the shoulders and backs of individuals who carry the raw hides onto ships in some South American ports. Infections of the face and neck appear to be the most dangerous; the mortality in such cases being 26 per cent, whereas infection of the extremities is rarely fatal.

Historical records contain almost countless instances in which references are made to the terrible havoc that this disease wrought in the sheep, cattle, and horse industries of the past ages. Kircher, a Jesuit monk, describes an outbreak which occurred among cattle in 1617 but later spread to man and claimed 60,000 victims among the latter. It still occurs in most sheep- and cattle-raising districts, but is more prevalent in some regions than in others. It is especially prevalent in Germany, France, Austria, Hungary, and Russia. It is stated that 72,000 horses died of this disease in Russia during 1864. In England and America the disease is relatively infrequent. The number of human cases varies with the number among the lower animals. Consequently, during the periods and in those districts where it is prevalent among domestic animals mankind suffers most. Bellon reported between 1909 and 1924, 205 cases that occurred in Marseilles among industrial workers. Twelve per cent of these were fatal. There are about 130 cases annually in the United States. These occur among laboratory workers, veterinarians, meat inspectors, farmers, cattlemen, butchers, and especially workers with wool, hair, and hides. There was an increase in the number of cases during the World War, 23 cases being reported from New York alone. These came mostly from the use of infected brushes, especially the shaving brush.

The anthrax micro-organism was the first pathogen to be seen under the microscope, as well as the first pathogen to be obtained in pure culture. Anthrax, likewise, was the first disease to be transmitted by inoculation to susceptible animals, and was the disease which Pasteur so dramatically demonstrated could be prevented by a vaccine. Bacteriological work on this disease was to transform it from one which threatened to mercilessly

and quickly devastate the entire cattle and sheep industry and before which man stood helpless and amazed into one which man can fully control. The net gain in dollars has run into the billions.

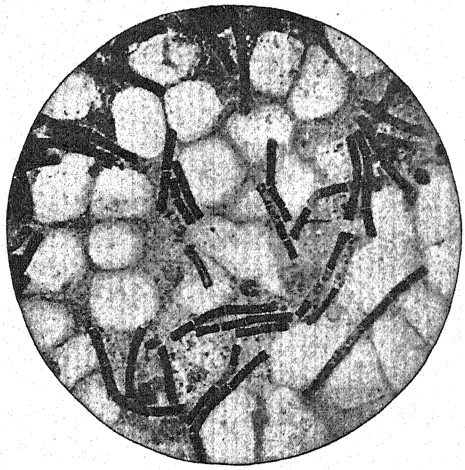


Fig. 138.—*Bacillus anthracis.* (*Fränkel and Pfeiffer.*)

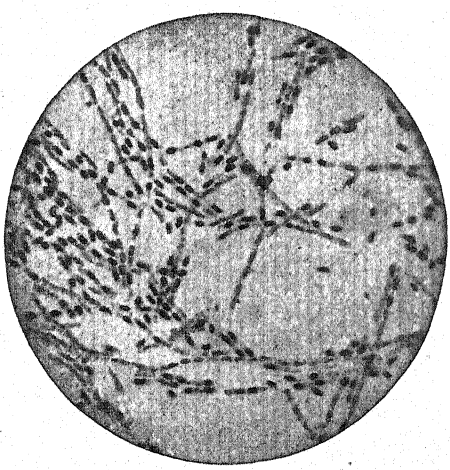


Fig. 139.—*Bacillus anthracis* with spores (× 1000). (*Fränkel and Pfeiffer.*)

The anthrax organism is a slender cylindrical nonmotile rod with squarely cut ends. It is from 1 to 3 microns in diameter and 5 to 10 microns in length. On artificial media and in the presence of oxygen it forms spores which are very resistant.

The spores do not form in blood until it is exposed to air; consequently, the bodies of animals which have died of the disease should be destroyed without letting their blood reach the soil. The spores withstand drying, strong germicides, and high temperatures to a much higher degree than most pathogens. In this respect it is similar to the botulinus and tetanus organisms. The anthrax bacilli can survive for years in soil, and upon hides which have passed through the tanning process; consequently, when the organisms once enter the soil of pastures they persist, although there is no evidence that they multiply in the soil. It was therefore once dangerous to graze cattle on pastures where the anthrax bacteria were present. Today, however, as a result of Pasteur's work, properly vaccinated cattle can be grazed on such pastures with impunity.

The prevention of anthrax in man rests primarily upon its eradication in domestic animals, and secondarily upon the disinfection of all products obtained from infected animals. As already mentioned, the bodies of animals that die of the disease should be destroyed without spilling their blood on the soil. Stables in which diseased animals have been kept should be thoroughly disinfected. Animals to be housed in such buildings or grazed on infected soil must first be vaccinated. Individuals dealing with infected material should use constant care not to infect themselves. This is accomplished by the wearing of rubber gloves in postmortems, and the disinfecting of hides, hair, and wool before they are worked into finished products for the use of man. Proper ventilation for carrying away dust where these products are being handled goes far in the protection of the worker. The refuse from tanneries, woolen mills, and the like should be properly disposed of to prevent soil or water infection.

Glanders.—Glanders is a communicable disease of horses, mules, and asses. Goats, rabbits, and dogs may contract it in the natural manner. It is readily inoculated into the ordinary laboratory animals, with the exception of the white mouse which is said to be immune. Cattle are immune. Man is quite susceptible and may contract it either from his fellowmen or from the horse, which is more usually the case. It has a high mortality in both man and the horse, but it is said to be most fatal in man when contracted from other human beings. Robins reported that of the 156 cases of glanders in human beings which he investigated, the percentage of recovery was found to be less than 6 per cent.

The infection enters the body either through abrasions in the

skin or the mucous membrane. It is characterized by the formation of inflammatory nodules in the nose or skin. The first form is known as glanders; the second, farcy. The nodules break down leaving a crater-like ulcer. Glanders may be acute or chronic. In acute glanders there is generally fever, a discharge from the nose, and a degree of prostration out of all proportion to the clinical signs; a generalized pustular eruption is very common. Death occurs in a week or ten days. In the chronic form there may be coryza, multiple subcutaneous and intramuscular abscesses, often associated with enlargements of the lymphatic glands and vessels; nodules may form in the mucosa of

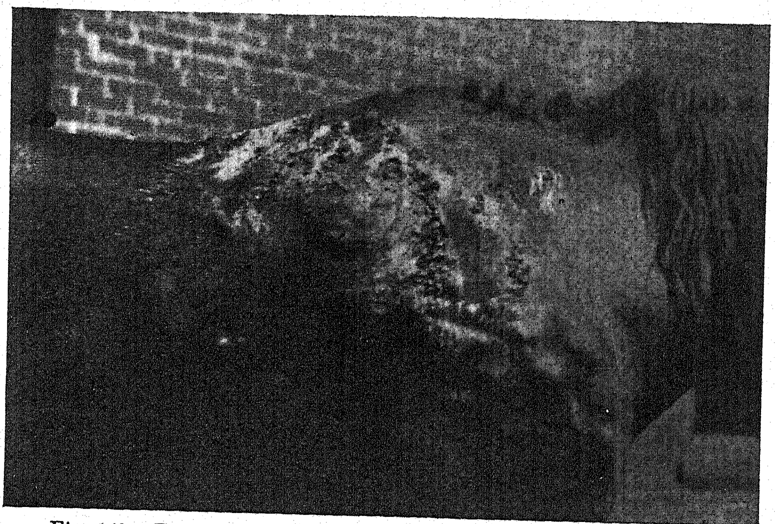


Fig. 140.—Farcy affecting skin of shoulder. (*U. S. Dept. of Agr.*)

the respiratory and alimentary tracts and may break down and ulcerate; necrotic foci may appear in the bones, and nodular lesions in the viscera. The disease may remain active for weeks, months or even years. It is usually, but not always, fatal. Gaiger, who was in the Indian Civil Veterinary Department, gives his experience with the disease which he contracted from an infected Arabian pony. The symptoms appeared March 4, 1911, and by June 25, 1913, the last lesion had healed. In the treatment he underwent no less than 45 operations, of which 27 were done under general anesthetics. One of the major operations was for the amputation of the right arm.

The cause of glanders, *Actinobacillus mallei,* is a rod-shaped

micro-organism with rounded or pointed ends. It measures from 0.3 to 0.5 micron broad and 1.5 to 5 microns long, is nonmotile and does not form spores. It stains feebly with watery solutions of the basic aniline dyes, but deeply with Löffler's methylene blue or carbol fuchsin. With the last two it assumes a striated appearance. It grows slowly on laboratory media. Its optimum temperature is that of the human body. It is not very resistant to heat or antiseptics. However, it can withstand drying for ten days and may survive for one or two months in water. Due to this property it is often spread in horses' drinking troughs. There is no evidence that it naturally multiplies outside the body of animals. Its prevention in man rests upon

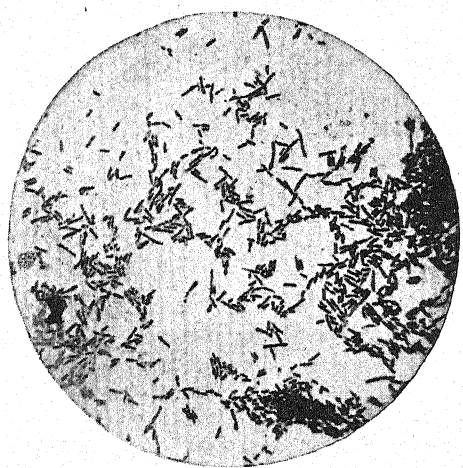


Fig. 141.—*Actinobacillus mallei.* (*Fränkel and Pfeiffer.*)

its prevention in the horse. The use of the automobile has very materially reduced the number of human cases.

Malta Fever.—A disease known as Malta fever has existed on the Island of Malta and elsewhere about the Mediterranean for years. More recently it has appeared in several other countries, including the United States. It is primarily a goat disease and is contracted by man from this animal, mainly through the use of infected milk, and occasionally through wounds.

It is characterized in man by headache, sweating, pains in the joints, arthritis, constipation, enlarged spleen, and irregular fever, and may persist for weeks or even months. The mortality is less than 2 per cent.

Bruce discovered the cause to be a micro-organism, now designated as *Brucella melitensis*. He did not explain the mode of infection. This was in doubt until 1905, when 65 goats, all apparently in healthy condition, were shipped from Malta to the United States. The raw milk was drunk during the passage by the captain and a number of the crew with the result that many of them contracted Malta fever. On arriving in America, *Brucella melitensis* was found in the milk of several of the goats, and the blood of 32 of them gave a positive agglutination test with the organism.

Evans gives the following general description of *melitensis*, the type species of *Brucella*.

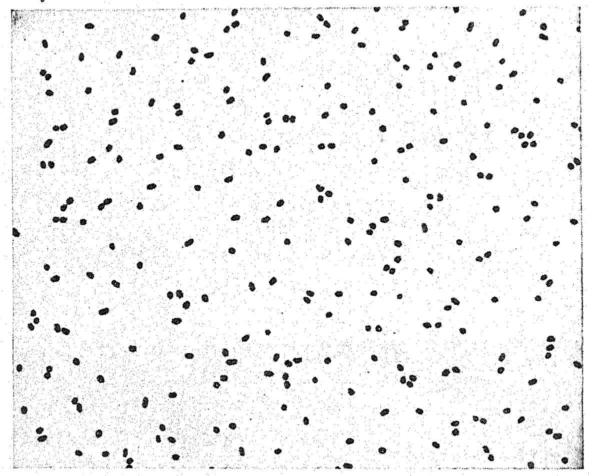


Fig. 142.—*Brucella melitensis*. (*From Ford, A Text-book of Bacteriology.*)

"Minute rods with many coccoid cells (the cells of two-day cultures grown on the surface of plain agar and stained with carbol fuchsin appear about 0.5 of a micron wide and 0.5 to 2 microns long); not forming endospores; nonmotile; aerobic, or preferring a slightly reduced, partial pressure of oxygen; without gelatin liquefaction; gram-negative; parasitic, invading animal tissue; neither gas nor acid production from the carbohydrates." The organisms withstand cold well, and are said to be able to survive in dust for many months.

In 1918 Miss Evans called attention to the close relationship between *Brucella melitensis* which caused Malta fever and *Br. abortus* which was first described by Bang in 1897. The two or-

ganisms are so similar both morphologically and physiologically that it is necessary to make an agglutinin absorption test in order to differentiate the one from the other. She further suggested the possibility of human infection with bovine strain of *melitensis* and in 1924 the first case of human infection with the abortion organism was recognized. When it is known that contagious abortion in cattle is world-wide and secondary only to tuberculosis in importance it can be readily seen that a new interest was created in undulant fever. It is becoming a disease of first magnitude. Each year new cases are reported from all parts of the United States and foreign countries.

It has been shown that from 0 to 100 per cent of samples of market milk examined carry the abortion organism and Fleischner and Myers report that the abortion micro-organism "is for practical purposes, always present in certified milk produced in the San Francisco Bay regions."

When it is known that the United States Public Health Service estimates that 30 per cent of the herds in New York State are affected with contagious abortion, and this is possibly a conservative estimate for the country at large, and there is a high probability of the organism occurring in most market milk, it is evident that human immunity must be fairly high or the disease usually occurs in a mild unrecognizable form. In man the disease is characterized by irregular and intermittent fever, consequently the name undulant fever. There are several strains of the *Br. melitensis* and those from goats and hogs are apparently more virulent for man than are the bovine strains.

The fact that *Br. melitensis var. abortus* may cause disease in man makes it advisable: (1) That individuals who handle diseased animals guard against infection. (2) That inasmuch as the porcine strain is the one most pathogenic for man, cattle and hogs should not be kept in close contact with each other. (3) That the disease should be controlled in cattle to as great an extent as possible. (4) It must be kept clearly in mind that the ultimate control of these diseases in man rests upon their eradication in goats, cattle, and hogs, and that the close cooperation between the veterinarian and health worker is a necessity for success, and (5) that all milk, even certified, be properly pasteurized before using.

Two general methods have been proposed for the control of contagious abortion among animals: (1) Control by vaccination and (2) control by agglutination testing and the segregating of reactors. There is considerable diversity of opinion as to the

value of vaccination and as to whether living or dead vaccines should be recommended. Most states have in effect some scheme for the control of contagious abortion, and it is the exception to find a state which does not have laws requiring the blood testing of all cattle shipped into the state.

Foot-and-mouth Disease.—Foot-and-mouth disease is an acute, highly communicable disease attacking chiefly the cloven-hoofed animals. Among human beings it occurs most frequently in children and usually results from the use of raw milk from diseased animals. However, it can result from the transfer of other infectious secretions. In cattle it is characterized by lameness due to lesions about the hoof, small vesicles in the mouth, loss of appetite, great emaciation, and marked diminution in the quantity of milk secreted. The mortality is low, but the economic loss is high due to impaired nutrition of the animal and the reduction in the milk flow. There is apparently no lasting immunity; consequently, once introduced into the herd the influence is long manifest. In human beings the disease is characterized by fever, sensation of heat and dryness in the mouth followed by the eruption of vesicles distributed over the lips, gums, edge of tongue, more rarely on the fingers and seldom on the toes. The disease is usually quite mild, although its results may occasionally be fatal in sickly or very young children.

Epidemics of the disease have occurred in various parts of the world and it is endemic in many countries at all times. It has been introduced into the United States on eight different occasions: Into the Eastern States in 1870; into Massachusetts in 1880 and 1884; into New England in 1902; into Detroit in 1908; into Chicago in 1914; into California in 1924; and into Texas in 1925. Each time it has been stamped out by the quick, systematic, cooperative work of local and federal officers. Often this has necessitated the destruction of whole herds of infected cattle with great economic losses. Consequently, quarantine is rigorously enforced. Infected manure, hay, utensils, drinking troughs, railway cars, animal markets, and barnyards; all may contribute in the spread of the virus.

The virus will pass the finest porcelain filters, as was shown by Löffler and Frosch in 1898. In April, 1924, Frosch demonstrated before the Microbiological Society of Berlin an exceedingly small bacterium which he had succeeded in photographing with the aid of the ultraviolet ray. He had cultivated this organism on both fluid and solid media, claimed to have successfully infected guinea-pigs, and caused mild symptoms in cows.

He has proposed calling the organism *Löffleria nevermanni.* A British and also a German commission so far have failed to confirm these findings.

Tularemia.—McCoy in 1911 described a plague-like disease which occurred among the ground squirrels of Tulare County, California. The following year he and Chapin succeeded in obtaining from diseased squirrels and growing on an egg medium a very small rod, 0.3 to 0.7 micron in length and often capsulated. The organism is found in large numbers in the spleen of the animals that have died of the disease. To this organism they gave the name of *Pasteurella tularensis.* It is killed at

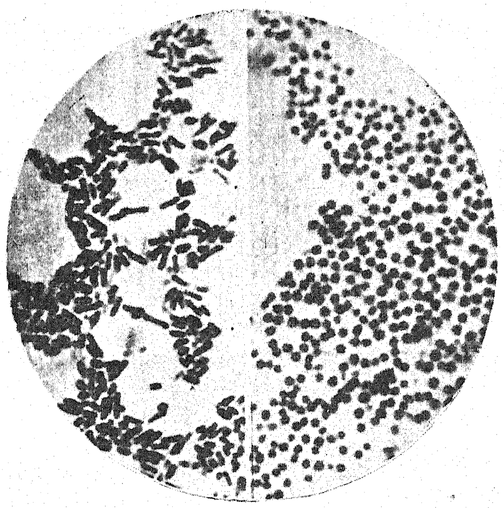


Fig. 143.—*Pasteurella tularensis* showing bacillary form (left) and coccoid form (right). (*From Edward Francis, Army Medical Museum, No. 37203-A.*)

56° to 58° C. in ten minutes, but withstands drying and cold for comparatively long periods. It is readily killed by formaldehyde, cresol, and the usual germicides.

Since the discovery of tularemia, according to Zinsser, it has been found in every state, except Maine, Vermont, and Connecticut. In nature, it is an infection of the ground squirrel and wild rabbits and hares of the Rocky Mountain states; of wild rats and wild mice in some parts of California; of quail, sage hens, and grouse in Minnesota; of sheep in Idaho; of wild rabbits in Japan, Norway, and Canada; of water rats in Russia; and of sage hens, grouse, and wild ducks in California and Mon-

tana. It also attacks man, causing a typhoid-like disease. It is transmitted among animals and also to man by blood-sucking insects, chiefly ticks and flies. Insects, however, are not essential to its spread, as a number of laboratory workers have been infected possibly in some cases through undamaged skin. It can also be transmitted by eating improperly cooked tissues of diseased animals. This occurred in four cases in Lee County, Virginia, three of which were fatal. A number of cases have been reported in which the primary seat of infection was the eye into which the infectious material had been mechanically transferred from rabbits or ticks. It has been suggested by Parker and Spencer that infection may take place by inhalation where laboratory workers are exposed to animals with extensive pulmonary involvement. There is no evidence that it tends to spread from man to man.

It has been shown that *Pasteurella tularensis* can be hereditarily transmitted in the intermediate host, the woodtick, from parent insect to eggs, nymphs, and larva. This makes the difficulty in its control greater than if infection came to the tick only by the biting of infected animals.

The prevention rests upon the care exercised in the handling and use of infected animals. Extreme care should be taken when dealing with susceptible animals in districts in which infection is prevalent. Rubber gloves should be worn in the dressing of wild rabbits, and every precaution taken to see that the animals to be eaten are not diseased, and even if they are not infected to cook the meat thoroughly.

CHAPTER XLV

SOME INSECT-BORNE DISEASES

It has been observed for centuries that certain so-called "contagious diseases" are peculiar in their geographical distribution, seasonal prevalence, and erratic methods of spreading. Numerous theories were evolved to account for this, but the true explanation did not come until the brilliant work of Smith, Manson, Finlay, Ross, and a score of others had been carried on in connection with what we now recognize as insect-borne diseases. The beginning of this new period in preventive medicine dates back to 1893 when Theobald Smith demonstrated that Texas fever, a disease of cattle, is transmitted only by the bite of a tick. Since then, many other diseases have been shown to be transmitted in a similar manner. These discoveries have placed in the hands of health workers means of subduing and in some cases of eradicating plagues which in the past had devastated the most fertile regions of the world and claimed millions of victims.

Classes of Insect-borne Diseases.—There are two classes of insect-borne diseases, namely, the biological, and the mechanical. The biologically transmitted diseases are directly dependent upon some biting or sucking insect for transmission. It is in the body of this insect that certain definite stages of the infective agent's life cycle are passed. So far as known, these infective agents have no other means of transmission. Usually the transmitting agent is limited to a specific species, or to a very limited number of nearly related species of insects, but whatever the cause may be these insects when they are once infected remain so throughout their lives. The infecting agent, so far as is known, belongs to the animal kingdom. It is evident that if the infecting agent passes a part of its life cycle in the insect, there must elapse a certain incubation period between the time when the insect becomes infected and the time when it is able to transmit the infection. This period of noninfectivity is known as the extrinsic period of incubation and represents the time necessary for the infective agent to complete its sexual cycle in the insect and reach the appropriate point of exit. Usually the insect becomes infected by feeding on some infected animal.

In a few diseases, like Texas fever, Rocky Mountain spotted fever, and African relapsing fever, hereditary infection may occur. Some of the more common biologically transmitted diseases are:

Disease	Cause	Insect
Malaria	*Plasmodium malariae*	Anopheles mosquito
Rocky Mountain spotted fever	*Dermacentroxenus rickettsi*	Tick
Texas fever	*Babesia bigemina*	Tick
Typhus fever	*Rickettsia prowazeki*	Louse
Yellow fever	A filterable virus	Aëdes mosquito

The mechanically conveyed diseases are incidentally transmitted by insects. The insects act as would an infected needle. They are not essential to the propagation of the virus. The infecting agent may be upon or within the body of the insect and is usually bacterial in nature. There is no incubation period in the body of the insect, and the transmission is not necessarily limited to a single species of insects. The more common members of this group of diseases are:

Disease	Cause	Insect
Cholera	*Vibrio cholerae*	Flies
Dysentery	Bacteria and amebae	Flies
Plague	*Pasteurella pestis*	Fleas
Typhoid	Typhoid bacilli	Flies
Tularemia	*Pasteurella tularensis*	Tick, deerflies

Characteristics of Insect-borne Diseases.—Although insect-borne diseases may occur in epidemics, they are never world-wide in their distribution, for it is evident that they are limited to those districts in which the insect and the infecting agent prevail. This gives these diseases a seasonal variation which is dependent upon the prevalence of the insect. They also vary with rainfall and temperature, for these determine the distribution of the insect. However, the temperature governs not only the occurrence of the insect, but in some of the biologically transmitted diseases it also determines whether or not the infecting agent can pass its life cycle in the body of the insect. It is known that insects do not migrate great distances of their own

accord, but they are occasionally mechanically transferred long distances by modern vehicles of transportation. Consequently, the infecting agent is usually distributed to various quarters of the globe by man where, if appropriate insects occur, the disease may become epidemic. We shall now consider in some detail malaria as a type of the biologically transmitted disease, and plague, as a type of the mechanically transmitted disease.

MALARIA

Historical.—For ages malaria has held a large proportion of the population of the globe in its bondage. There appear in the writings of Aristophanes (about 422 B. C.) clear descriptions of the disease, and Hippocrates states that men who drink marsh waters get enlarged spleens, and those who live in low, marshy districts are neither tall nor well built, but dark-colored and wanting in courage and endurance. This reflects the belief that was held even to a comparatively modern period. It is reported that Empedocles (about 550 B. C.) subdued the disease in a city in Sicily by draining its swamps. The first step in its conquest, however, probably came about 1660 when Countess d'El Cinchon, wife of the Viceroy of Peru, was cured of malaria by a concoction made from the bark of a native tree. She spread this knowledge among Europeans, and for two hundred years there was a difference of opinion concerning the therapeutic value of this agent. In some cases it wrought miraculous cures, while in other cases it was without effect. Today we understand that what seemed failures which occurred in many cases were due to the fact that fevers other than malaria were being treated. In 1820 Pelletier and Caventou extracted from the cinchona bark the active constituent, since known as quinine; but it was not until 1880 that Laveran, a French army surgeon, announced the discovery of the malaria parasite in the human red corpuscles of persons ill with the disease. But even this discovery was not sufficient to make possible the control of the disease. This did not take place until 1895 when Ross proved that the Anopheles mosquito is responsible for the spread of the disease, and today we know that the control of the disease rests upon the destruction of the mosquito and the use of quinine.

Prevalence.—Malaria has played a leading rôle in the history of the nations. It is held by Jones and others that the fall of Greece, and particularly of Rome, was due in a large measure to malaria, which was introduced into Rome with the slaves from Africa. The colonization of tropical Africa and Central America

has been determined by malaria. It has been estimated that it is even at the present time the direct or indirect cause of over one half of the entire mortality of the human race. Even today there is no other disease which compares with it in importance. Sir Ronald Ross holds that India alone loses over one million persons annually from this disease, a greater number of deaths than resulted during the first two years of the World War. Malaria claims its victims by the thousands in Africa, Southern Europe, South and Central America. Even the southern part of our own country loses approximately twelve thousand persons annually from this disease. Dr. Howard estimates that there are annually three million cases in the United States and it is believed by some that malaria is one of the most potent reasons why the South is backward, for malaria not only kills, but it

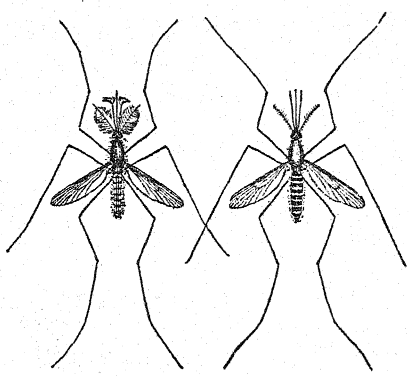


Fig. 144.—Anopheles mosquitoes. (*Howard.*)

also devitalizes. It has been estimated that malaria, in one form or another, places an annual tax of $14 an acre on all tillable land in Georgia and $4 an acre on all land in Louisiana. Dr. Howard also makes the astonishing but well-founded statement that the annual financial loss to the United States from malaria is not less than $100,000,000.

Malarial Parasite.—The malarial parasite belongs to the animal kingdom. Three species are now recognized, which are associated with three distinct types of the disease: *Plasmodium vivax,* causing tertian fever, which is comparatively mild and rare; *Plasmodium malariae,* causing quartan malaria, which is common and likely to relapse; and *Plasmodium falciparum* causing estivo-autumnal malaria, which is most malignant and pernicious in tropical places. These organisms pass their sexual

life in the body of the mosquito, and their asexual life in the body of man. The sporozoites are present in the saliva of infected mosquitoes and are injected into the human being when

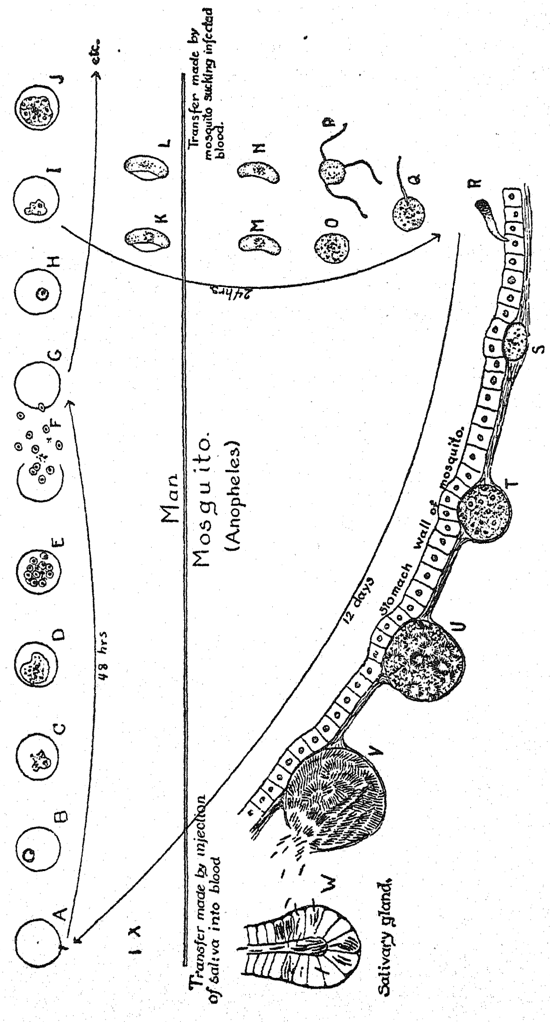


Fig. 145.—Life history of malarial parasite. (*After Chandler.*)

the insect bites and withdraws blood. On entering the blood the sporozoites grow at the expense of the red corpuscles and possibly also obtain nutrients from the plasma. In its youngest recognizable stage, the malarial parasite appears to be attached

to the corpuscle as a small, glassy, oval, signet-ring-like body with ameboid movements. It proceeds to burrow slowly into the substance of the corpuscle, and increases in size at the latter's expense. On attaining its maximum development, which varies in size with the species, the parasite undergoes a segmentation of its cell substance, giving rise first to a rosette or mulberry-like body within the corpuscle and leading eventually to the formation of small, round spores, or merozoites. These are liberated by the ultimate disintegration of the corpuscles. On being set free, they fasten themselves to new red corpuscles and begin their cycle of development again.

The cycle from one generation to the next varies with the species of the malarial parasite and may be as short as twenty-four hours. The bursting of the corpuscles and the release of the poisonous products coincide with the characteristic chills and fever of malaria. This rapid multiplication may give rise to enormous numbers of the parasites in the blood. The actual quantity in the human body in a case of severe estivo-autumnal malaria has been estimated at 600 cc., or over one pint. This is approximately 3,000,000,000,000 malarial parasites, a number fifty times greater than the seconds which have elapsed since the birth of Christ! As long as the blood of the host is a suitable environment, they continue to multiply in regular manner; but when conditions become unfavorable due to the production of antibodies in the blood of the patient, they develop special sexual forms, or gametocytes, male and female, in the form of sausage-shaped crescents, and remain in this form until sucked up by the mosquito. If the temperature is right these gametocytes pass through a complex sexual phase in the digestive tract of the mosquito. The female gametocyte is fertilized by the male, elongates, and becomes quite like a little worm. This finds its way between the inner and outer lining of the stomach and here develops spores. At times a single capsule may contain 10,000 spores, and there may be 500 capsules on one mosquito's stomach. In about twelve days, depending upon the temperature, after the infected blood has been taken by the mosquito, the capsules are mature and rupture, and the spores find their way into the saliva of the mosquito and from here into the blood of the human host when bitten by the mosquito.

Method of Spread.—Malaria is spread solely by the bite of the Anopheles mosquito. Over one hundred species of this insect have been described, but less than half of these have been proved to harbor the malarial parasite. It forages out-of-doors

in the twilight. This is often taken advantage of by individuals living in malarial districts. They may bite at any hour of the day indoors; consequently, careful screening is essential in malarial districts.

The Anopheles mosquito breeds in almost any standing water, provided it contains microscopic organisms on which it can feed. The eggs are deposited on the surface of the water and float separately on their sides. In a day or two these hatch into larvae or "wiggle tails." These ordinarily rest and feed just below and horizontal with the surface of the water. In about a week they develop into curiously shaped creatures known as pupae. These pupae have no mouths; consequently, they do not feed but are air-breathing animals and remain near the surface of the water. In two or three days there emerges from these pupae the adult mosquitoes, the imagoes.

Prevention.—The control of malaria rests upon either the destruction of the malarial parasites in man by the use of quinine, or the destruction of the mosquitoes. The latter method is most effective, and depends first of all upon the destruction of the natural breeding places of the mosquitoes. The manner in which this method is to be carried out varies with the locality. At times it means the draining of swamps, at other times, the filling in of low places. Where neither of these methods is feasible the stagnant water may be oiled or at times planted with fish. The fish feed upon the mosquito larva, and under favorable conditions are very effective in cutting down the number of mosquitoes which is all that is essential in the control of malaria as it is not necessary to completely eradicate the mosquito.

PLAGUE

Plague is primarily a disease of the rat and secondarily of man. Often rats die in great hordes during plague epidemics. Plague is generally transferred from rat to rat and from rat to man by the flea. In man it takes three forms: (1) *Bubonic Plague*—This form of the disease is spread by the flea. The bite usually occurs on the legs, and the germs in attempting to spread are caught by the lymph nodes, in this case in the groin. These swell and often suppurate. The swollen glands are known as "buboes" and the disease as the "bubonic plague." The mortality varies, but is usually from 40 to 70 per cent. (2) *Pneumonic Plague*—This form is spread by contact. The lungs are involved and the mortality is nearly 100 per cent. (3) *Septicemic Plague*—In this numerous hemorrhages occur under the

skin which turns black. This has given rise to the term "the black death." It is transmitted by the bite of the flea. Although there is a tendency for each form to run true, yet, occasionally, the one form changes to the other and when once changed to the pneumonic the spread is rapid.

Both sacred and profane history abounds in references to plague. Quickly and with a great fury it has swept time and again over large areas of the civilized world. Tacitus describes the plague days of 68 A. D. in these words: "Houses were filled with dead bodies, and the streets with funerals; neither age nor sex was exempt; slaves and plebeians were suddenly taken off, amidst lamentations of their wives and children, who while they assisted the sick or mourned the dead, were seized with the disease, and perishing were burned on the same funeral pyre." When the plague days in Rome (80 A. D.) were at their height the victims in this city of 1,000,000 numbered 10,000 a day. The plague constantly smoldered in the cities of Europe and Asia and periodically burst into a raging demon. The plague of 1348 claimed 25,000,000 victims in Europe, one fourth of the total population. During this epidemic the population of Rome was reduced to 20,000. In the face of such a devastating plague we are not surprised to find Boccaccio writing concerning the plague days in Florence: "Such was the cruelty of Heaven and perhaps of man, that between March and July following, it is supposed and made pretty certain, that upwards of 100,000 souls perished in the city alone, whereas, before the calamity it was not supposed to have contained so many inhabitants. What magnificent dwellings, what noble palaces were depopulated to the last persons, what families extinct, what riches and vast properties left, and no heir to inherit; what number of both sexes in the prime and vigor of youth—who in the morning, Galen, Hippocrates, or Aesculapius himself would have declared in perfect health—after dining heartily with their friends here have supped with their departed friends in the other world."

During the Middle Ages it struck with terrific fury in many parts of Europe, sweeping everything before it, leaving confusion, panic, and death in its trail. And even in more modern times it has been trailed, time and again, across the American continent. Even today it requires constant, careful vigilance to keep it from flaring up in Europe and America. In India it still claims its victims by the millions.

The Cause.—During 1894 Kitasato and Yersin, working independently of each other and almost simultaneously, obtained

from the buboes the *Pasteurella pestis*. Since then this has been repeatedly done by others, thus establishing the principle that the organism always occurs in the body of the diseased. The evidence that it is the specific cause has been furnished by several laboratory accidents which have resulted in the plague. The organism is a short, thick rod with well-rounded ends varying in length from 1 to 2 microns, and in diameter from 0.5 to 1 micron. It shows great variation in shape and is prone to pro-

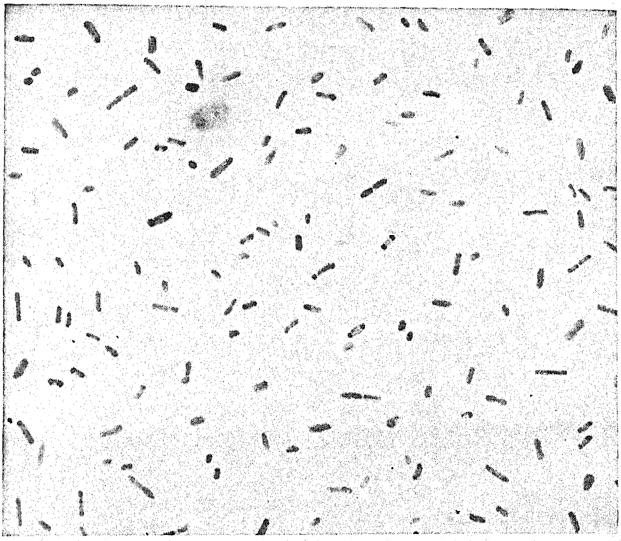


Fig. 146.—*Pasteurella pestis*. (*From Ford, A Text-book of Bacteriology.*)

duce involution forms, especially when grown on salt agar (agar containing 3 per cent sodium chloride). When properly stained the two poles are deeply colored, whereas the central part remains unstained. It is nonmotile and produces no spores although under appropriate conditions a capsule can be demonstrated. It is aerobic and grows best at a temperature of about 30° to 37° C. It is quite resistant to cold but is readily destroyed by heat, drying, and the ordinary disinfectants. The plague bacillus is readily isolated from infected tissue by rubbing the material on the shaved area of a guinea-pig. The skin of the guinea-pig acts as a differential filter and permits the plague bacilli to enter but keeps out other organisms. Consequently, the bacillus may be obtained in pure culture from the body of the infected animal.

Method of Spread.—Plague, as already mentioned, is primarily a disease of the rat and secondarily of man. It is also prevalent among some other animals, as for example, the ground squirrel in California, and the marmot in certain parts of Tibet. The transfer of the virus from the rat or other animal to man is made mechanically by the flea. The ingested blood may be regurgitated by the flea into the bite, or more often the virus traverses the alimentary canal of the flea and is deposited with the feces, and this rubbed into the skin by scratching. The pneumonic form is spread by direct contact.

Fig. 147.—California ground squirrel. (*Museum of Vertebrate Zoölogy, University of California.*)

Prevention.—It is impossible to exterminate the flea, as all who have lived in flea-infested districts know; but it is possible to get rid of the plague-infected rats and ground squirrels. A systematic vigorous campaign has been waged for years in California against infected ground squirrels, with the result that the plague is kept within bounds. Campaigns against rodents which keep the plague alive should be vigorously pushed, for in the case of the rat it is estimated that annually it wastes and destroys property in the United States to the value of $167,000,000. Moreover, the rodents are known to convey, in addition to plague,

by one means or another the following diseases: Acute infectious jaundice, food infections, trichina, tapeworm, other parasites, and rat-bite fever.

Rodents may be destroyed by trapping, poisoning, destruction of breeding places, the keeping of dogs or cats which prey upon them, and the building of rat-proof storehouses for grains and other things on which they feed.

CHAPTER XLVI

THE FILTRABLE VIRUSES

THE work of the last decade of the nineteenth century brought into prominence a group of infecting agents capable of passing through extremely fine filters. Consequently, they were referred to as filtrable viruses. In the early years of their discovery none of the viruses had been seen with the microscope; consequently, the term "ultramicroscopic" was used synonymously with filtrable. More recent work, however, has developed methods which render a number of the filter-passing viruses visible. Yet, even today there are numerous filtrable viruses which remain in the domain of the invisible. Whether their invisibility is due to minute size or composition is not clear, nor is the biological relationship of the various members known; nevertheless, the term "filtrable virus" is a convenient one to use when reference is made to the heterogeneous group of filter-passing infecting agents. They are probably corpuscular in makeup and possess the characteristics of living matter. They are considered by some authorities to be living entities, whereas others believe them to be certain forms of self-reproducing enzymes.

History.—The first systematic study of a filtrable virus was made by Pasteur on rabies in 1881, but it was not until 1892 when Iwanowski in the course of his investigations of the mosaic disease of tobacco demonstrated that there were filter-passing viruses pathogenic for plants. This Russian investigator found that he could produce mosaic disease in healthy plants by inoculating them with the filtered juice from diseased leaves. Six years later Beijerinck published confirmatory evidence of these findings, and during the same year Löffler and Frosch published their epoch-making studies on foot-and-mouth disease. These investigators found that they could produce the disease by inoculating animals with the filtrate made from the contents of the vesicles occurring on the mouths of diseased animals. They passed this from animal to animal, each time causing the disease, thus demonstrating that the specific causative agent multiplies within the body of susceptible animals. Since 1898 many diseases have been shown to be due to filtrable viruses, but as yet

there is no absolute agreement among authorities as to all the diseases which should be classified as caused by filtrable viruses.

Following is a list of the majority of filtrable virus diseases as given by Park and Williams.*

Neurotropic Diseases:
- Acute anterior poliomyelitis
- Rabies
- Epidemic encephalitis
- Acute disseminated encephalomyelitis
- Postvaccinal encephalitis
- Disseminated sclerosis
- Japanese encephalitis
- Pseudorabies (dogs, cats, cattle)
- Spontaneous encephalitis in rabbits
- Borna disease (horses, cattle)
- Guinea-pig paralysis
- Salivary gland disease of guinea-pigs
- Louping ill (sheep)
- Fowl plague
- Fowl paralysis

Neurodermotropic Diseases:
- Variola
- Vaccinia
- Varioloid
- Paravaccinia
- Alastrim
- Varicella
- Herpes simplex
- Herpes zoster
- Swinepox, goatpox
- Sheep-pox, horsepox
- Cowpox
- Virus III (rabbits)
- Foot-and-mouth disease (cloven-footed animals)
- Vesicular stomatitis (horses, cattle)
- Infectious pustular stomatitis of horses
- Fowlpox

Dermotropic Diseases:
- Molluscum contagiosum
- Common warts

Respiratory Diseases:
- Rubeola
- Rubella
- Parotitis
- Psittacosis
- Common colds
- Influenza ?
- Pertussis ?
- Rinderpest (cattle)
- Hog cholera
- Guinea-pig epizootic
- Equine influenza
- Distemper (dogs)

Insect-borne Diseases:
- Yellow fever
- Dengue fever
- Sandfly fever
- Nairobi disease of sheep
- Catarrhal fever of sheep
- African horse sickness

Neoplastic Diseases:
- Myxomatosis of rabbits
- Leukema of chickens
- Rous' sarcoma (fowl)

Diseases in Fish:
- Lymphocystic disease
- Carp-pox
- Epithelioma of barbus

Diseases in Insects:
- Sacbrood of bees
- Polyhedral ("wilt") diseases

Diseases in Plants:
- Mosaic diseases — affecting tobacco, potato, cucumber, lettuce, cabbage, sugar cane, and other plants

Diseases of Bacteria:
- Bacteriophage — a transmissible lytic disease, affecting many species of bacteria and exhibiting a partial specificity

* From Park and Williams, "Pathogenic Microorganisms," Lea and Febiger, Publishers.

Nature of Filtrable Viruses.—Inasmuch as the criterion used for admission to this group is the ability to pass a fine filter, it is evident that it comprises a very heterogeneous group. Beijerinck in his early work concluded that the virus which caused the mosaic disease of tobacco was not corpuscular, but was of the nature of a self-propagating enzyme. This same idea is held and vigorously defended by many modern authorities in respect to the bacteriophage. However, the facts point to the conclusion that the viruses are corpuscular, that they multiply, pass some filters but not others, tend to settle out of solution on standing, and can be concentrated by centrifuging their solution. They are very minute, for in the light of certain evidence that we have at hand, the virus causing chicken plague, for example, must be smaller than a molecule of hemoglobin which is less than 23 to 25 mμ.

Although there is still a great deal of debate as to whether or not the viruses are living entities, all the leading authorities recognize in the viruses the characteristics of living organisms for they multiply, are susceptible to conditions which are injurious to living things, and possess the power of adapting themselves to their surroundings. Plants or animals which have been attacked by them and recover possess a high and lasting immunity; this is even more pronounced than in the case of bacterial disease. They apparently are obligate parasites, for up to the present time, with one or two exceptions, they can be cultivated only in living tissue. They usually have an affinity for specific cells and in the diseased animal these cells contain certain cell inclusions which are characteristic of the specific disease. The relationship of these cell inclusions to the specific disease is not understood. Some authorities consider them to be the specific causative organisms, whereas others look upon them as deteriorations of the cell due to the action of the specific virus.

The biological nature of the filtrable viruses is at present largely conjecture. The fact that some possess a definite life cycle and are resistant to glycerin but susceptible to saponin and bile, points to their protozoan character. But according to Simon:

"To assume, on the basis of our present knowledge, that the filter passers are either bacteria or protozoa, is unwarranted. We may say that certain observations suggest that a few protozoa, and possibly bacteria may assume a filter-passing size, but in so far as the great majority of the filtrable viruses go there

is no evidence whatsoever to warrant the claim that their taxonomic position has been established. It might possibly be best to classify them as protista, were it not for the fact that there is evidence to suggest that some of them may be inanimate even though corpuscular. Theoretically, there can certainly be no objection to the formulation of the hypothesis that disease-producing agents of corpuscular nature may exist, which in part belong to the lowest forms of life, which are neither bacteria nor protozoa and in part to the higher forms of ferments, and that the main group of filtrable viruses may properly find its position here rather than among either the bacteria or protozoa."

The epidemiology of virus diseases is not greatly different from that of bacterial diseases. Some, for example smallpox, are extremely contagious; others like poliomyelitis, are of low infectivity. Carriers play a prominent part in both bacterial and virus infections. The virus may enter the body by different portals: Rabies enters through skin abrasion, hog cholera, sacbrood, and polyhedral disease through the intestinal route. It is not quite definitely known whether foot-and-mouth disease enters through the upper nasopharyngeal area or through the intestinal tract. Diseases are often transmitted from sick to healthy individuals by means of insect vectors, including lice (typhus fever), ticks (Rocky Mountain spotted fever), sandfly (sandfly fever), mosquitoes (yellow fever), and aphids (plant mosaics). In the case of some virus diseases, the insect vector is highly specific. Typhus fever, trench fever, and dengue fever are believed to be almost absolute. In the mosaics, the spread may be due in some cases to a specific aphid, whereas in others disease may be spread by several species.

Immunity in some of the bacterial diseases may be produced by killed cultures of the causative organisms, but many authorities argue that immunity in the case of the filtrable viruses can be produced only through the use of living viruses. It is even claimed that immunity endures only so long as the virus remains alive in the immunized individual. However, in many cases immunity is high and of long duration. Three of the more important diseases of man, which have been shown to be due to filtrable viruses, are dealt with in this chapter.

SMALLPOX (VARIOLA)

Smallpox is as old as the human race and was the first communicable disease controlled by means of a vaccine. It is conveyed from man to man by the rather intimate exchange of

secretions of the nose and mouth, and is due to a filter-passing agent which up to the present time has not been propagated outside the human body except in certain experimental animals. In the epithelial cells of affected areas of diseased animals appear round or oval bodies named after their discoverer, Guarnieri bodies, which vary in diameter from 1 to 8 microns. The discoverer considered these the causative organism, but many authorities believe them to be merely cell inclusions or degenerations resulting from the action of the virus or its toxins. Similar inclusions occur in the related disease, cowpox. The ones causing cowpox occur in the cytoplasm, whereas those of smallpox occur in the nucleus. This, together with the fact that an attack of cowpox gives an immunity to smallpox and that smallpox may be transformed into cowpox, has given rise to the idea that they are two manifestations of the same disease. Smallpox virus when inoculated into cattle does not grow well at first, but eventually becomes transformed, so that it cannot be distinguished from cowpox. Furthermore, after it is once transformed into cowpox, it cannot be changed back into smallpox.

Vaccination.—Long before the practice of vaccination was discovered, there originated somewhere in the Orient the practice of inoculation. The Chinese took scabs from smallpox patients, ground them up, and blew them into the nostrils. The Turks inserted material from smallpox pustules into scratches made in the skin of persons to be immunized. Lady Mary Montague introduced the practice into England in 1717, and during the remainder of the century this method of inoculation was extensively resorted to in the control of smallpox. The disease thus produced was the real smallpox and could spread from one individual to another. It therefore caused an increase in the number of cases, and occasionally it had a fatal termination. However, for some reason yet unexplained the infection was less severe than that contracted in the usual manner. Through inoculation the mortality which prior to its use occasionally reached 50 per cent was reduced to less than 1 per cent.

It had been the belief in pastoral districts, for how long we do not know, that an attack of cowpox protected against smallpox. Benjamin Jesty, a Dorsetshire farmer, acting on this belief, rubbed some of the material from cowpox eruptions into scratches in the skin of his wife and his two infants and thus rendered them immune to smallpox. But it was Jenner who first placed vaccination upon a sound basis, when in 1796 he took pus from a cowpox pustule on the hand of Sarah Nelms and introduced it into

the arm of a lad, James Phipps. Six weeks later the boy was deliberately exposed to smallpox and even inoculated with the virus. He was found to be immune, and today it is universally agreed by authorities that the control of smallpox rests upon vaccination.

Various methods have been used for the introduction of smallpox virus into the body of the individual to be immunized, such as by puncture, scarification, scratch, or incision. The method recommended by Dr. Rosenau is the scratch or incision method. A scratch about ⅛ inch long is made with a sterile needle, and a drop of the vaccine is rubbed into it. The best place to do this is on the arm, but the spot chosen must be thoroughly washed with soap and water followed with alcohol or acetone before the scratch is made.

Value of Vaccination.—The value of vaccination has been amply demonstrated. Before the days of Jenner, everyone expected to contract smallpox; now no one expects to, but the unvaccinated occasionally do. In Germany, Japan, and other places where vaccination is made compulsory and is systematically carried out, smallpox has disappeared from the native inhabitants, but in unvaccinated districts smallpox still claims its victims. In such districts it often requires an outbreak of the disease in order to force the inhabitants to avail themselves of the protection which science offers. It is generally agreed today by authorities that the unvaccinated individual is not only endangering his own health, but he is withholding from the community a protection which rightfully belongs to it; and it is just as important in the 'land of the free' that vaccination be made compulsory as is education.

Dr. Rosenau summarizes the claims made for vaccination in the following manner:

"1. 'Duly and efficiently performed it will protect the constitution from subsequent attacks of smallpox as much as that disease itself will.'

"2. It protects against smallpox for a period which varies with the individual, but which for practical purpose may be taken to average seven to ten years.

"3. The protection may be renewed by repeating the vaccination.

"4. Persons vaccinated every ten years will be reasonably protected throughout life.

"5. Vaccination and revaccination systematically and generally carried out confer complete protection to a community or

nation. In other words, while individual protection is not always lasting, communal protection is absolute.

"6. A person vaccinated once and at a later time contracting smallpox as a rule has the disease in a less serious form than an unvaccinated person. The degree of favorable modification of smallpox depends upon the length of time elapsing between the vaccination and the attack of smallpox.

"7. The beneficial effects of vaccination are most pronounced in those in whom the vaccine affection has run its most typical and perfect course."

The best practice is to have children vaccinated when they are but a few months old; again when they enter school; and thereafter, at intervals of ten or more years or oftener if intimately exposed to smallpox.

Manner of Spread.—Smallpox is so infectious that it used to be taught that it was due to a "volatile virus," and consequently, spread through the air. However, authorities in general now agree that it is spread in much the same manner as other communicable diseases are. Discharges from the mouth and nose, the pus from the sores, and the scabs or scales from the skin are all known to be infectious. These may readily be transferred, either directly or indirectly from the ill to the nonimmune and cause the disease. The period of incubation is from nine to fifteen days, and the disease is communicable before the eruption occurs.

RABIES OR HYDROPHOBIA

Rabies is a disease primarily of the dog and other animals and is transmitted to man usually by a bite, although the mere licking of an abraded area of the body by a rabid animal is occasionally sufficient to transmit the disease. Pasteur proved it to be due to a virus occurring in the nervous tissue and saliva of the rabid animal. There occur in the nervous tissue of such animals cell inclusions known as Negri bodies. These may be the actual virus or body cells injured by the virus. The Negri bodies are of importance in that they furnish a ready method by which the bacteriologist can diagnose the disease.

Symptoms of Rabies.—There is much misunderstanding concerning the symptoms of rabies. The dog in the early stages does not manifest a furious raging disposition with a tendency to bite man or other animals, nor is there a stage in the development of the disease in which the animal shows any fear of water. In the early stages it may wade into it, and lap water with relish,

but it is evident this would not occur in the later stages on account of paralysis. A rabid dog manifests a condition of nervous unrest. It may travel considerable distances from its home. It attempts to hide itself, crawling under any object which may offer a temporary resting place. However, it cannot rest; it seems dissatisfied with everything. It is especially familiar with other animals, likes to be caressed by its master and if permitted will lick his hand. This is a very dangerous procedure for the virus may be in the saliva and if it gets into an abrasion is likely to cause the disease. Later the affected animal runs at an uncertain but rapid rate, and is likely to snap at anything, including man, which may cross its path. "Sooner or later the animal passes into the paralytic stage. Its lower jaw droops, the saliva accumulates in the mouth, and the animal attempts to remove it with its paws. The paralysis, beginning usually in the posterior extremities, gradually involves all the muscles of the body, and the animal dies from asphyxiation. During the paralytic stage, on account of the fact that the animal cannot close its mouth, it does not bite, but its saliva contains the virus in large amounts. Its effort to pull the thick mucus from its throat may lead a witness to believe that the animal has a bone in its throat and to attempt to assist in its removal. Such a procedure is accompanied by great danger."

Sometimes the animal may pass directly into the paralytic stage without the early stages of restlessness; this is known as "dumb rabies." In this form the animal lies partly paralyzed with its mouth open. Man often becomes infected from handling such animals.

Transmission of Rabies.—Rabies is transferred from the lower animals to man almost wholly by the saliva which finds its way into a bite or any injured tissue. The relative danger of infection varies with the part of the body bitten and the nature of the wound inflicted. Bites on the bare surface such as the hands and face, are far more likely to give rise to rabies than are those through clothes. This is probably due to the clothing keeping back much of the virus and certain areas are richer in nerves and nearer the central nervous system, hence, the virus which follows the nerves can reach a vital point. Deep, ragged wounds, such as those inflicted by the wolf and dog, are more dangerous than the contuse wound produced by the sheep or horse.

Virus.—The active principle of rabies is a filter-passing virus which occurs principally in the central nervous system and in the saliva of the afflicted animals. It is sensitive to light, drying,

and to many of the ordinary disinfectants, and is especially resistant to extreme cold. It is very sensitive to formaldehyde and nitric acid. The virus sometimes appears in the saliva as long as fourteen days before symptoms. Therefore a good rule to follow when bitten by a dog is to watch the dog for fourteen days, and if the animal does not develop rabies in that time the victim of the bite is safe. If it does, he should receive the Pasteur treatment immediately.

Prevention of Rabies.—The prevention of rabies in man is easy in some districts, but comparatively hard in others, for it rests principally upon preventing it in dogs. In areas like Russia, France, and the United States where the disease is prevalent among wild animals, which in turn transmit it to the domestic animals, the problem is much greater than in England. A control of the number of dogs is essential. This is accomplished by the destruction of stray dogs and the levying of a tax on all dogs kept. Some progress has been made in the immunization of the dogs against rabies. In Japan over one hundred thousand dogs have been immunized with a great reduction in the disease. The muzzling of dogs has eradicated rabies in England. This practice not only prevents the spread of the disease among domestic animals, but it prevents its conveyance to man and is the only effective method in a rabies-infected area.

The procedure which has reduced rabies from a comparatively frequent to a rare disease is the Pasteur treatment. This should be given in every case where individuals are bitten by rabid animals. In cases where the bite is from an apparently healthy dog it should be watched for fourteen days. If rabies develop in the dog during this time the patient should immediately receive the Pasteur treatment. Where the animal has been killed its head should be packed in ice and shipped to a bacteriologist prepared to make examination for Negri bodies.

MEASLES (RUBEOLA)

Measles is one of the most highly communicable diseases known. In this respect it resembles smallpox. It is peculiar to man and attacks every race, both sexes, and all ages. It is often considered a child's disease, because of the fact that most individuals in temperate zones contract the disease and thus gain a life-long immunity before they reach maturity. However, it must not be forgotten that this immunization process costs annually in the United States alone approximately twelve thousand lives.

History.—We owe our first accurate description of measles to the Arabian physicians of the tenth century. Probably prior to this period it was confused with smallpox. Since then we know that it has been endemic in most districts and periodically becomes epidemic, the epidemic dying out only when there are no more nonimmunes. Occasionally, it reaches virgin districts with direful results. The epidemic which swept the Fiji Islands in 1875 is an example. So far as known the disease had never touched the inhabitants of the island until that year. It was brought to them by the son of the king of the Fiji Islands, and through a conference that was held at this time, it was conveyed to all parts of the island. The population of the island numbered about 150,000; of these it is officially stated that 40,000 died of the disease. In some districts from 27 to 28 per cent of the population died.

The high mortality is usually attributed to the disease being carried into a virgin population where inherited immunity is not present. The more reasonable explanation appears to be: (1) The number ill at a given time was so great that there were not sufficient well to care for the ill. It is a well-known fact that proper nursing is the best safeguard against complications and death in measles. (2) The natives, ignorant of the disease, often bathed in streams and otherwise exposed their bodies while suffering with measles. This gave rise to dysentery and pneumonia which are prone to accompany the disease and are usually the cause of death. That this is the proper explanation appears from the fact that in certain garrisons where the natives received proper care no such mortality occurred; moreover, all epidemics among virgin populations have not shown a similar high mortality. Brownlee writes:

"I visited some years ago at Highland Glen from which measles had been absent for more than eighty years. An epidemic had just ended; it swept the glen from the western end to the eastern, hardly anyone—man, woman, or child—escaped the infection. It may be noted, by the way, in contradiction of the erroneous and quite common belief that though the soil was quite virgin in the epidemic not a single death occurred."

Probably the belief that the disease takes a more virulent form among a virgin population than among others must go the way of the other erroneous ideas, for example, measles is more likely to be fatal among adults than among children. Measles often greatly disorganizes armies where troops become infected, but

there is no evidence that it is more fatal to adults than to children.

Virus.—Each year brings new reports of the discovery of the causative organism of measles, but whether any of the recently described organisms are the specific cause is not certain. Measles is known to be due to a filtrable virus occurring in the secretions of the nose and throat. The virus is present even before symptoms appear. This accounts for the high communicability of the disease. In the early stages of the disease the virus occurs in the blood, and Anderson and Goldberger apparently communicated it to the monkey. The virus soon disappears from the blood and is probably not communicable after the fever has subsided. All evidence points to the conclusion that the scales are not infectious. The virus is frail and is soon destroyed by sunlight, desiccation, disinfectants, and heat.

Manner of Spread.—The high communicability of measles early led to the idea that the disease was spread in the air, but all evidence points to the conclusion that it is spread by rather intimate contact. Any method by which the secretions of the nose and mouth of one are quickly transferred to another favors its spread.

Immunity.—One attack of measles usually confers a lifelong immunity, although second attacks do occur. The observation of this high immunity has led within recent years to the use of a convalescent serum obtained from the individuals who have recently recovered from measles. This serum protects or else greatly mitigates the resulting cases of measles depending upon the time which has elapsed between exposure and the giving of the protective serum. If given in from one to four days after exposure only a few cases occur. There has resulted a passive immunity which disappears in a few weeks. If the serum is given at a somewhat later period, between the fifth and the eighth day, the result is a more or less modified attack of the disease, varying from an extremely trivial malaise to a typical attack of measles. Even in the latter case the disease is usually mild. In these cases an active immunity results, consequently it is recommended by some authorities that in healthy children over three years of age, and where the prevailing type of measles is mild, the administration of the serum should be deliberately postponed until the sixth or seventh day, in order that an active immunity may result.

Prevention.—Quarantine, the closing of schools, and the use of terminal fumigation, all appear to be without effect on the spread

of measles; consequently, the only thing that can be done is to use those well-established means of preventing complications and sequels. The care of the eyes during and just after an attack can do much in saving eyesight. Darkened rooms during the attack and no attempt to use the eyes too soon after a case of measles are good precautions. Measles predispose to pneumonia and tuberculosis; consequently, the convalescent should avoid individuals ill of these diseases, as well as all those conditions which contribute to them. Statistics indicate that the main mortality from measles occurs among the young, and that 90 per cent of the fatal cases occur during the first five years of life; consequently, when parents intentionally, or carelessly expose their children to the disease with the belief that the younger children have it in a light form, they are making a grave mistake. Furthermore, it should be remembered that the infectious diseases take not only the weaklings, but often the most vigorous and robust. This is well pointed out by Vaughan in the following passage:

"Even the great philosopher of modern science, Herbert Spencer, taught that infectious diseases, especially among children, were in the long run beneficial since they cut out in early life those who are unfit and give greater opportunity for the proper development of those who should live. Is there any basis in fact for this belief? Did smallpox during the centuries when it was uncontrolled by vaccination kill only weaklings and spare the robust? Do measles, scarlet fever, whooping cough, infantile diarrhea, play that function today? If these questions can be answered authoritatively in the affirmative, then we should hesitate in our attempt to eradicate the infectious diseases, especially those most prevalent and most fatal in infancy and childhood. No child acquires measles, whooping cough, or scarlet fever, unless exposed to the infection. The danger to the life of the individual child depends upon many conditions, especially upon the state of health at the time of the exposure and the amount and virulence of the infection transferred to the child at the time of exposure. Are these conditions determined or modified by the degree of fitness or unfitness to live possessed by the individual child? If a careless nurse carries articles of clothing soiled with discharges from a scarlet fever patient into a household where there are children, is there any reason for believing that the less fit of these children are more likely to come in contact, or are more likely to suffer from coming in contact, with the imported virus? An individual with open tuberculosis

deposits bacilli-laden sputum on the floor of a schoolroom filled with children in all degrees of physical fitness and unfitness. By what law or by what chance will the less fit of these children be infected, while the more fit escape? Badly infected milk may be used in a children's school. Some will drink more of the poisonous milk and some will drink less, and possibly a few will drink none of it. All will suffer or escape as they fall into one of these classes. What will lead all the unfit children to drink the poisonous milk and what will keep all the fit children from drinking any of it? If the fitness of a child to live is to be determined by passing through some such ordeal as exposure to a highly dangerous infectious disease, let us not leave this to nature but proceed to inoculate every child in a scientific and exact way. Suppose that we determine the minimum amount of white arsenic that may kill, say a weakling at one year of age; then on its first birthday we administer this dose of arsenic to every child. Are we to suppose that all those who might grow into inefficient or vicious citizens would die, while all those who are to develop into perfect manhood or womanhood are to live? If we are going to imitate nature in this experiment we must not weigh out the dose given each child, but measure it out, guess at it; and if we are going to imitate nature more accurately we shall select a blind person to measure out for each child its dose. The idea that the infectious diseases of childhood kill only the weaklings, or even kill the weaklings in larger proportion, is based upon a complete misunderstanding and misinterpretation of all epidemiological science. It would be just as sensible to attempt to improve the race by mustering all the children in a community and have them pass down a street while blind-folded men with repeating rifles fired into the crowd. Fortunately, even the preachment of Herbert Spencer on this point has in no way abated or hindered the attempts of the sanitarian to control and eradicate the infectious diseases prevailing at any period of life."

Every known means should be used in the prevention of communicable diseases, and it is well to constantly have in mind that the fundamentals on which prevention is founded are: (1) Communicable diseases are due to micro-organisms, and these are the descendants of other similar micro-organisms. (2) The great majority of microbes which cause disease in man multiply only in the body of man and the lower animals. (3) The overwhelming majority of all diseases are transmitted by direct contact. Their carriers are fingers, flies, and food. (4) A high state of bodily health should be the aim of all, but it does not neces-

sarily confer immunity to the communicable diseases. (5) In some diseases immunity may be acquired by a mild attack of the disease produced by vaccination; in others it may be transferred from one animal to another through the blood by means of so-called "antitoxins."

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